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SOME GENERAL ASPECTS OF PATHOLOGICAL CONDITIONS CAUSED BY FILTERABLE VIRUSES *

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In a previous paper an attempt was made to summarize and to correlate in a general way knowledge concerning filterable viruses. At that time, facts and hypotheses regarding the nature and characteristics of the agents themselves were primarily considered. During the discussion, however, it was shown that in the majority of virus diseases a close relationship exists between the etiological agent and cells of the host. In view of this intimate type of parasitism, it seems desirable at the present time to examine carefully and, if possible, to correlate information regarding the reaction of host cells to viruses. Moreover, a knowledge of the pathological conditions produced by viruses is essential to the study of this group of etiological agents, because their existence cannot be determined, nor can their identification be established in any manner other than by the evidences of their activity exhibited in some host.

In spite of the fact that many viruses appear incapable of multiplying in the absence of suitable living host cells, it is not definitely known whether their reproduction occurs intra- or extracellularly. Nevertheless, these agents have a profound influence upon cells and produce within them remarkable changes. This influence most likely accounts for the fact that in lesions produced by many viruses the intracellular changes are sufficiently characteristic to be spoken of as inclusion bodies. In this respect a number of virus diseases differ from those caused by ordinary bacteria.

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The inclusions have attracted the attention of many workers whose ideas concerning their nature have led to numerous discussions. It is undoubtedly true that these bodies are interesting and play an important rôle in experimental and diagnostic work, yet there are other pathological phenomena that are just as interesting as, and perhaps more important than the inclusions themselves, inasmuch as a better knowledge of them may lead to a clearer understanding of the nature of the action of viruses on cells and consequently to an explanation of why certain diseases exhibit significant inclusions while others do not. In view of the fact, however, that inclusion bodies are thought of immediately when one mentions the pathology of virus diseases, a summary of the knowledge concerning these structures will be given first and then some of the other interesting features of the pathological conditions induced by viruses will be discussed.

Inclusion Bodies

Inclusion bodies have been seen in cells of plants, insects, fish, birds, and mammals affected by virus diseases. In some of the diseases the bodies are intranuclear, *e. g.*, in varicella and in polyhedral diseases of caterpillars; in others they are found in the cytoplasm, *e. g.*, in vaccinia and in mosaic diseases of some plants; in still others they occur both in the nucleus and in the cytoplasm, *e. g.*, in smallpox and in paravaccinia. Many of the inclusions described, however, cannot be accepted as specific or characteristic and it is these that detract from the significance of the ones well established and accepted by numerous critical observers. In Table I are listed the majority of diseases in which inclusions of one kind or another have been described. The diseases are grouped according to the location of the inclusions in affected cells.

In spite of the chaos suggested by Table I, no worker familiar with the microscopic pathology of virus diseases doubts the importance and significance of Guarnieri bodies in vaccinia, Negri bodies in rabies, Bollinger bodies in fowl-pox, polyhedral bodies in certain diseases of insects, and the nuclear inclusions seen in varicella, herpes, and several other virus diseases. Since some of the diseases in the table exhibit inclusions of significance while others do not, I have made a selection of the pathological conditions in which the inclusions appear sufficiently characteristic to be of importance. The

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TABLE I

A List of the Majority of Diseases in which Intracellular Inclusions have been Described. The Diseases are Grouped According to the Location of the Described Inclusions within Affected Cells.

A. CYTOPLASM	Mosaic disease of certain plants
	Sheep-pox
	Contagious epithelioma (fowl-pox)
	Molluscum contagiosum
	Lymphocystic disease of fish
	Rabies
	Distemper of dogs
	Fowl plague
	Lethargic encephalitis
	Trachoma and inclusion blenorrhea
	Hog cholera
	South African horse sickness
	Rickettsia diseases
	Measles
	Scarlet fever
	Cancer (malignant growths)
	Kurloff bodies (guinea pigs)
	Todd bodies (frogs)
	Grahamella (moles)
	Bartonella bacilliformis (verruga peruviana and Oroya fever)
	Bartonella muris (splenectomized rats)
	Protozoan-like bodies in white blood cells of fowls in Palestine and Nigeria
B. NUCLEUS	Polyhedral disease of certain caterpillars
	Foot-and-mouth disease
	Vesicular stomatitis
	Borna disease
	Virus III infection of rabbits
	Herpes zoster
	Salivary gland disease of guinea pigs
	Epithelioma of fish
	Carp-pox
	Warts of *Discoglossus pictus* (frog)
	Warts
	Condyloma acuminatum
	Psoriasis
	Protozoan-like bodies observed in human visceral lesions of unknown etiology
C. CYTOPLASM AND NUCLEUS	Smallpox (and alastrim)
	Cow-pox (vaccinia)
	Paravaccinia
	Chicken-pox
	Infectious myxomatosis of rabbits
	Symptomatic herpes

selected diseases have been placed in Table II and grouped according to the location of the significant inclusions within affected cells. It is not unlikely that other diseases will be added to the table, and that some now included may in the future be omitted. In Plates 22 and 23 the significant inclusions of the diseases listed in Table II are graphically portrayed.

Nature of Inclusions

Various ideas are held concerning the origin and nature of inclusion bodies, and, in a general way, they may be divided into three groups. By some investigators they are considered merely as products of degeneration, but by others they are believed to be the virus itself, while by yet others they are thought of as virus surrounded by a mantle of altered cellular material.

Inclusions as Virus Itself: The idea that inclusions represent the virus itself is not absolutely irrational, inasmuch as bacteria and protozoa are frequently found within cells. Moreover, some protozoa are obligate parasites and multiply only in the cytoplasm or only in the nucleus of suitable host cells, while others reproduce both in the cytoplasm and in the nucleus of such cells. In fact, observations concerning coccidia, malarial parasites, and the more recently described organism of Wright and Craighead have in the main been responsible for the idea that inclusions are parasites.

Inclusions as Virus Surrounded by Altered Cellular Material: Upon the discovery that the etiological agents of the diseases under discussion pass through earthenware filters, a group of workers immediately realized that inclusion bodies probably do not represent virus alone, inasmuch as many inclusions (2–15 microns in diameter) are sufficiently large to render such a possibility unlikely. To adapt theories to facts, von Prowazek then described his hypothesis concerning the nature and development of viruses and their relation to inclusions. According to this worker, extracellular forms of a virus, "elementary bodies," are from 0.25 to 1.0 micron in diameter and are able to pass through filters. Upon entering a cell the "elementary bodies" become "initial bodies" which immediately begin to reproduce by division, thus forming a colony of parasites within its host. The cell then reacts to the presence of these minute organisms around which a mantle of altered cellular material is thrown. In this manner von Prowazek's *Chlamydozoa*, mantled

TABLE II

A List of the Filterable Virus Diseases in which Intracellular Changes are Sufficiently Characteristic to be of Significance. The Diseases are Grouped According to the Location of the Significant Changes within the Cells.

A. CYTOPLASM	Mosaic disease of certain plants	
	Sheep-pox	
	Cow-pox (vaccinia)	
	Contagious epithelioma (fowl-pox)	
	Molluscum contagiosum	
	Rabies	
	Lymphocystic disease of fish (no reports on filtration)	
	Infectious myxomatosis of rabbits	
B. NUCLEUS	Polyhedral disease of certain caterpillars	Inclusions are acidophilic
	Symptomatic herpes	
	Herpes zoster — No reports on filtration.	
	Chicken-pox — No reports on filtration.	
	Virus III infection of rabbits	
	Salivary gland disease of guinea pigs	
	Borna disease	
C. CYTOPLASM AND NUCLEUS	Smallpox (and alastrim)	
	Paravaccinia (no reports on filtration)	

Rickettsia diseases are not included in the table because the evidence is in favor of the idea that there is a distinct difference between rickettsiae and the inclusions discussed in this paper.

After further study, carp-pox, epithelioma of fish, and warts of *Discoglossus pictus* (frog) may be placed in Group B in view of the inclusions (probably acidophilic) described in nuclei of affected cells.

Evidence is increasing in favor of the idea that the nuclei of cells affected by warts and condyloma acuminatum show certain characteristic changes — basophilic masses or "chromophane" masses of Lipschütz.

Trachoma and inclusion blenorrhea are omitted from the table awaiting further observations concerning the nature and significance of the cytoplasmic inclusions observed in affected cells.

After further study some of the diseases in the table may be removed or new ones may be added.

animals or inclusion bodies, are formed. The host cell finally ruptures freeing the parasites which again become "elementary bodies." In general, von Prowazek's ideas are in accord with those of Lipschütz, who suggests for the small bodies without mantles the name *Strongyloplasmen*, rounded bits of protoplasm. The ideas of these men are plausible enough, yet in most instances it is difficult either to prove or to disprove conclusively whether they correctly portray the actual facts concerning viruses.

Inclusions as Products of Cellular Degeneration: At present, numerous investigators believe that inclusions do not consist of virus and that at least a major portion of the bodies comprises products of cellular degeneration. These workers frankly admit, however, that in most cases, it is very difficult to establish the fact that the virus is not enveloped by the products of cellular reaction. In one instance only has it been possible to show that active virus is not structurally related to the inclusions characteristic of the disease in which they occur. This was accomplished by Glaser, who found that polyhedral bodies observed in virus diseases of caterpillars can be separated from active incitant and when freed from it are incapable of producing disease in normal larvae.

Although many workers consider inclusions as products of cellular degeneration, there is no unanimity of opinion regarding the manner in which they arise and the cellular constituents they comprise. The nuclear inclusions seen in several diseases, *e. g.*, varicella, herpes, and Virus III infection of rabbits, resemble each other so closely that a differentiation of the diseases one from another by means of the appearance of the inclusions alone is impossible. More specificity, however, is observed concerning cytoplasmic inclusions, inasmuch as no two virus diseases exhibit absolutely identical changes in the cytoplasm of affected cells. The marked degree of specificity displayed by these inclusions is believed by Cowdry and others to be due to the fact that the cytoplasm, by virtue of its composition and position in the cell, may respond more readily and more characteristically to different kinds of stimuli arising either intra- or extracellularly. In spite of the tremendous variations exhibited by inclusions of different diseases, the constancy of their size, form, staining reactions, location in cells, and components such as proteins, fats, and lipoids, in any one disease under similar conditions is very striking. This constancy, however, is by no means dependent upon a

homogeneity of the inclusions, for the majority of them comprise several kinds of constituents. A complete review of the ideas concerning the origin and structure of characteristic inclusions is not possible at the present time. Nevertheless, a few opinions will be cited for the purpose of emphasizing the radical manner in which views of competent workers differ.

*Vaccine Bodies:** Guarnieri (1892) believed vaccine bodies to be protozoa, and, since he thought of them as possessing a peculiar power of devouring the cytoplasm of cells, thus creating a hole within which they lie, gave them the name *Cytoryctes vacciniae*. Although Hückel (1898) considered Guarnieri bodies specific for vaccinia, he believed that they are not the virus itself, but arise entirely within the cytoplasm of affected cells. According to him, under the stimulus of the virus, a portion of the cytoplasm undergoing colloid degeneration near the nucleus becomes cyanophilic (blue-staining). Later the erythrophilic (red-staining) cytoplasm in the immediate neighborhood of the blue mass undergoes hyaline degeneration and separates from the rest of the cell. The two masses, the blue in the center surrounded by the red, are situated within a "hole" in the cytoplasm and constitute a vaccine body. Ewing (1904–05) believes that Guarnieri bodies are altered portions of the cytoreticulum into which nuclear material has diffused. According to Cowdry (1922) these bodies are the result of a stimulation of cells by vaccine virus leading to an increase of a substance present in small amounts in normal cells.

Molluscum Bodies: Molluscum corpuscles were first described in 1841 by Paterson, who thought of them as parasites. In 1892, Macallum (Plate 24, Fig. 5) stated that molluscum bodies are merely migrated plasmosomes or modified chromatin arising as a result of hyperplasia and hyperchromatosis. According to him, one might classify the disease as a neoplasm. Lipschütz (1911) believes that three abnormal substances are found in the cytoplasm of cells affected by molluscum virus; migrated nuclear substance, products of a keratin-like degeneration, and a mass of virus, elementary bodies or *Strongyloplasma hominis* (Plate 22, Fig. 24). In 1918, Sanfelice, using Mann's stain in the study of molluscum bodies, described the following steps in their formation. Normal cells swell. Nucleoli re-

* Weigert, 1874, first described and portrayed inclusions in cells affected by smallpox virus. (Plate 23, Fig. 8.)

tain the red instead of the blue stain and migrate into the cytoplasm where they again stain blue. In the cytoplasm the nucleoli become vacuolated and granular, and, as they enlarge, assume the appearance of typical molluscum bodies filled with fine red-staining granules. More recently, 1927, Goodpasture expressed his ideas concerning molluscum bodies in the following manner: "The bodies are not derived from extruded nucleoli, nor from any formed cytoplasmic constituent. . . . The minute bodies develop about, and later within, cytoplasmic vacuoles which may be regarded as the cellular response to the presence of a living foreign body . . . elementary bodies of Lipschütz. . . ."

*Bollinger Bodies.** Bollinger, in 1873, described in tissues affected by fowl-pox or epithelioma contagiosum a hyperplastic condition of epithelial cells, many of which were greatly swollen, 25 microns in diameter, and contained near their nuclei large bodies with fat-like appearances. These bodies were considered by Bollinger to be parasites. Michaelis (1904) also found evidences of proliferation and swelling of cells in this disease. The inclusions, according to him, are made up of albuminous and fatty substances. Although he was unable to determine whether the disease is parasitic or not, he was, nevertheless, of the opinion that the inclusions themselves do not represent parasites. Borrel (1904), using Loeffler's flagella stain in the study of material from the lesions of fowl-pox, found myriads of minute coccoid bodies about each of which appeared some kind of capsule. Burnet (1906) thought that the small bodies described by Borrel represent the incitant of the disease and are closely associated with the specific inclusions. Ludford and Findlay (1926) state that the inclusions of fowl-pox appear only in epidermal cells, an occurrence which is probably related to the process of keratinization. They state further (1) that the earliest indication of the activity of the virus on cells is the formation in their cytoplasm of small vacuoles, to the periphery of which minute granules adhere, (2) that mitosis† is usually seen in cells containing such vacuoles, (3) that

* Rivolta, 1869, was probably the first investigator to describe inclusions in fowl-pox.

† In Fig. 7, Plate 24, mitosis is taking place, and, although Ludford and Findlay say nothing regarding the matter, one gets the impression from the picture that the virus body has divided also and that a daughter body will go with each daughter cell. This phenomenon has attracted practically no attention in virus diseases of animals, yet it is a well-known fact (Kunkel and others) that inclusions in mosaic diseases of

the vacuoles increase in size, and coincidentally with the enlargement become enclosed by a lipoidal lining and exhibit a granular appearance internally, (4) that many cells become hypertrophied and at an early stage in the disease show a complete reversal of the Golgi apparatus, and finally (5) that the inclusions are not the virus itself. (See Plate 24, Figs. 6–9.)

Negri Bodies: In nerve cells injured by the virus of rabies Negri (1903) discovered certain inclusions which he regarded as protozoa. Levaditi and his coworkers (1926) still believe in the parasitic nature of Negri bodies and propose that they be named *Glugea lyssae.* According to Acton and Harvey, however, these structures are not parasitic, but arise as a result of an interaction between the cytoplasm of cells and particles of nuclear matter which have been extruded through catabolic changes induced by the action of the virus. In Goodpasture's opinion (1925), Negri bodies represent the results of a slow necrobiosis of nerve cells and originate in a degenerative change in mitochondria producing vacuoles with small bodies within them, "about which through a partial disintegration of neurofibrillar material a capsule is formed."

Nuclear Inclusions: Ideas of the nature and origin of acidophilic nuclear inclusions have also led to numerous discussions. Loewenthal believes that they are parasites. Lipschütz, Goodpasture, and others are of the opinion that the bodies, although not necessarily consisting entirely of virus, are in some manner intimately associated with it. Luger and Lauda, however, contend that these nuclear changes are due to oxychromatic degeneration (Plate 22, Figs. 8–15) and have no genetic affinity with the virus.

Sufficient examples have been cited to show that able investigators frequently disagree radically in their views concerning the inclusions of each virus disease. Furthermore, from what has been said it is obvious that the intracellular structures described in various morbid conditions may not be of a similar character. Therefore, in discussing intracellular pathology, one should be careful not to make general statements based on observations limited to one virus

certain plants frequently divide when the host cells do and that a daughter inclusion body goes with each daughter cell. This phenomenon is interesting, but it does not necessarily imply that the inclusion is an autonomous parasite, inasmuch as the Golgi apparatus and at times mitochondria (Cowdry) divide during cell division, and a portion of each structure goes with each daughter cell.

TABLE III

A List Indicating Hypotheses Concerning the Nature and Origin of Inclusions Observed in Cells Affected by Viruses

A. CYTOPLASMIC INCLUSIONS

1. Bacteria.
2. Fungi.
3. Protozoa.
4. Products of cellular degeneration or cellular reaction to the viruses. According to Lipschütz and others, the etiological agents are visible; they are called strongyloplasms, initial bodies, or elementary bodies. According to Prowazek and others, inclusion bodies consist of viruses surrounded by products of cellular reaction; they are called chlamydozoa (mantled animals).
5. Products of cellular degeneration; agent unseen or not identified; no proof of genetic relation between inclusion and virus.
   (*a*) Engulfed or phagocyted cells undergoing degeneration.
   (*b*) Nucleoli or necrotic nuclear derivatives extruded into the cytoplasm.
   (*c*) Extruded nuclear material mixed with cytoplasmic elements.
   (*d*) Central body or archoplasmic structures undergoing degeneration.
   (*e*) Degeneration of cytoplasm and cytoplasmic structures.
   (*f*) Degeneration of daughter nuclei in cells in which amitotic division of the nuclei occurs.
   (*g*) Results of secretory activity of affected cells.
   (*h*) Inclusions consist of substances normally present in small amounts in the cytoplasm. Under the influence of viruses, however, these substances are greatly increased.

B. NUCLEAR INCLUSIONS

1. Protozoa.
2. Similar to No. 4 under Cytoplasmic Inclusions.
3. Nucleoli undergoing degeneration.
4. Results of nuclear degeneration (oxychromatic degeneration). Inclusions are not genetically related to the viruses.
5. Similar to (*h*) under No. 5 (Cytoplasmic Inclusions) except that the changes occur in the nucleus instead of in the cytoplasm.

disease. For convenience, a summary of the ideas regarding the mode of origin and the nature of inclusions is given in Table III.

CHANGES PRODUCED IN INCLUSIONS BY EXPERIMENTAL PROCEDURES

The relation of smallpox to vaccinia furnishes an interesting topic for discussion. In most respects these diseases behave differently: one is highly contagious, the other is spread only by direct inoculation; in one, generalized lesions regularly occur, in the other, this

happens only rarely; in cells affected by smallpox, inclusions are seen in the nucleus as well as in the cytoplasm, in cells injured by vaccinia, significant inclusions are found only in the cytoplasm. Smallpox virus, however, passed through several calves, becomes vaccine virus. Furthermore, it remains vaccine virus, even though it be returned to human beings. Thus, smallpox virus passed from man to lower animals, excepting monkeys, is so altered that apparently a different disease is caused by its action. Not only is the character of the disease altered but the intracellular pathology is also changed. Observations of a similar nature have been made in regard to rabies. When "street virus" is passed through a large number of rabbits a "fixed virus" is obtained which no longer produces typical inclusions but causes atypical Negri, lyssa, or passage bodies. These facts are extremely interesting, and certainly, so far as smallpox and vaccinia are concerned, no one has been able to determine just what occurs when smallpox virus becomes vaccine virus. Nor is it known why the intracellular pathology under these conditions is also altered.

Inclusions in Malignant Growths

Paterson saw molluscum bodies in 1841, Bollinger described inclusions in contagious epithelioma in 1873, and Guarnieri observed vaccine bodies in 1892. In spite of these facts, the chief interest in inclusion bodies during the latter part of the nineteenth and the first part of the twentieth centuries centered around peculiar intracellular structures observed in cells of neoplasms. Some of this interest may have been due to the fact that many workers considered molluscum contagiosum and epithelioma contagiosum as tumors. Aside from that, however, great interest was evidenced regarding the so-called cancer inclusions described by Plimmer, Feinberg, von Leyden, Russell, Schüller, Thoma, Darier, and others.

Practically all inclusions described in tumor cells have been situated in the cytoplasm. Many of them have been portrayed as having definite and characteristic structures, *e. g.*, Plimmer bodies. Hypotheses regarding the nature of cancer inclusions are numerous, and strange to say, with a few exceptions, are similar to the theories in Table III concerning cytoplasmic inclusions in virus diseases. Interest in these structures in cancer resulted in little or no progress in the recognition of the cause of newgrowths. Furthermore, the

irregularity of their occurrence, and the difficulty encountered in finding them, rendered these bodies of little value as a diagnostic aid. Consequently, cancer inclusions are rarely mentioned at the present time.

Concurrently with a decreasing interest in cancer bodies and a better understanding of the nature of such diseases as contagious epithelioma and molluscum contagiosum the inclusions in virus diseases received an increasing amount of attention. The regularity with which characteristic structures are found in cells affected by certain morbid conditions render them of value in experimental and diagnostic work, *e. g.*, in rabies and in smallpox. Jackson and Goodpasture's descriptions of nuclear changes observed in duct cells of guinea pigs' salivary glands induced Cole and Kuttner to search for a virus. Their investigations resulted in the discovery of a new filterable virus which injected into susceptible pigs produces nuclear changes identical with those described by Jackson and Goodpasture. Moreover, all work with polyhedral diseases of caterpillars is controlled by the presence or the absence in blood cells of characteristic nuclear changes. Consequently, it is unlikely that the inclusions in virus diseases will cease to be of interest as quickly as did those of tumors. It is true that the nature of the intracellular bodies has not been definitely determined. Nevertheless, in spite of the ignorance concerning their nature, inclusions have held and will continue to hold an important position in the study of this group of diseases.

Many attempts to produce significant inclusions by artificial means have been unsuccessful. It is interesting, however, to note that, when intracellular changes resembling inclusions of virus diseases have been experimentally induced, they have followed the use of such agents as arsenic, tetanus toxin, and diphtheria toxin. Some of the experimentally induced changes closely resemble those seen in virus diseases, yet they are not identical. Therefore, under properly controlled conditions, the presence of inclusions, accepted as significant, will undoubtedly in the majority of instances be indicative of the presence of a virus in the immediate vicinity.

Pathological Changes Other than Inclusion Bodies

In the foregoing section of the paper, facts and conjectures regarding inclusion bodies have been discussed. While these intracellular structures play an important rôle in the study of virus diseases, they, nevertheless, constitute a minor part of the reaction in the host to the infectious agents. Furthermore, if no inclusions are found in cells affected by certain disease processes, or if the intracellular changes described have not been generally accepted as significant, one is not warranted in concluding that the morbid conditions are not caused by viruses. This statement is emphasized by the fact that significant inclusions have not been described in measles, poliomyelitis, and fowl plague, diseases generally believed to be caused by filterable viruses. Therefore, one is justified in raising the question as to whether inclusions constitute the only characteristic response of cells to viruses. Moreover, one would like to know whether, in addition to specific or characteristic responses, there are also reactions similar to those induced by substances of bacterial or other origin.

Inflammation

Although the majority of pathologists interested in virus diseases have devoted most of their energy to the study of inclusions, there is a group of workers who either ignore the existence of such structures or believe that they are of no significance, and contend that the pathology of certain virus diseases is not essentially different from that observed in inflammatory processes produced by ordinary bacteria. Furthermore, as one reads the reports of work recorded in the literature, one is impressed by the fact that many investigators assume that inflammation caused by bacteria is thoroughly understood. Of course, such is not the case, inasmuch as it is not known whether certain responses of the host are due to the direct action of bacteria and their products, whether they are dependent upon the presence of substances derived from host cells that have been injured or killed by bacterial activity, or whether they are caused by the action of an injurious agent formed or liberated when infectious organisms (antigen) unite with their specific antibody.

The fact that inflammation occurs in many virus diseases cannot be denied, and, despite the acute nature of some of the diseases, if

secondary infections do not intervene, the inflammatory process is usually characterized by an infiltration of mononuclear cells. The question whether the inflammation is a primary or secondary phenomenon, however, has in certain instances led to lengthy discussions. Weigert (1874) looked upon the primary changes caused by the virus of smallpox as non-inflammatory and considered them to be necrobiotic or diphtheroid in nature. According to him, when the primary degenerative changes in the epidermis have reached a certain stage in their development, the inflammatory reaction appears as a secondary phenomenon and consists of exudation of fluid into the area of degenerated cells, of proliferation of epidermal cells with giant-cell formation around the necrotic tissue, and of infiltration of various kinds of leucocytes. Auspitz, Unna, Renaut, Ledingham, and others have regarded the variolous changes in the skin from the beginning as merely the expression of an acute inflammatory process. Furthermore, Unna and Ledingham have raised the question whether the virus of smallpox must necessarily cause necrosis of epidermal cells and whether the necrobiosis frequently observed is only accidental or secondary to an inflammatory reaction which begins in the corium. According to this view, the degenerative changes in epidermal cells and the formation of pocks result from pressure caused by the accumulation of exudate just beneath the epidermis.

In a number of diseases, particularly those involving the skin, it is difficult to determine precisely the location and the character of the primary injury or to follow accurately the development of a lesion. This difficulty is experienced because of the complex nature of the skin and the rapidity with which the lesions of such diseases as measles, smallpox, and varicella develop. If the inciting agent is borne into the skin by the blood, it is highly probable that the initial injury is in or around small blood vessels just beneath the epidermis, provided the cells in these tissues are susceptible to the virus.

So far as the discussion in the present paper is concerned, it is not so important to determine the point of the primary lesion caused by the virus as it is to ascertain the nature of the injury. Frequently conditions are unfavorable for such investigations. In some diseases, however, and under certain conditions in others, information concerning this question may be obtained. For instance, in variolous or vaccinal lesions of a rabbit's cornea definite and characteristic

changes are observed in the epithelial cells before any evidence of inflammation in the form of cellular or other exudate is seen. The lesions of molluscum contagiosum occur within the epidermis, and according to Benda,* Unna,* and Goodpasture (1927) little or no inflammatory reaction is observed in the corium. Furthermore, if inflammation occurs accidentally through secondary infection or as the result of treatment, the lesions heal promptly (Henderson, 1841). Goodpasture (1925) working under experimental conditions, found that the first evidences of injury caused by rabic virus are observed "within ganglion cells, not in the surrounding tissue," and that "these cells may undergo complete necrosis without cellular or other exudate about them." He also found that Negri bodies frequently "occur in great numbers within the ganglion cells entirely in the absence of evidences of inflammation in the form of cellular or other exudation." Finally, the pathological picture presented by mosaic disease in plants is one of necrosis and hyperplasia, and that observed in bacteriophagy is unlike the morbid processes usually associated with inflammatory diseases.

Degeneration and Proliferation

If inflammation, as it appears to be, is a secondary phenomenon in many virus diseases, what, then, are the primary changes produced in cells by these active agents? In all probability, they are either degenerative or proliferative in character. In fact, both types of changes are usually seen, and it is difficult at times to determine definitely whether degeneration precedes proliferation or whether they occur in the reverse order.

In certain diseases, particularly the ones producing vesicles in the skin, the degenerative changes have attracted most attention and the majority of workers consider that they precede evidences of proliferation. Opinions as to the type of degeneration, however, are numerous and many names have been used in its description, *e. g.*, coagulation necrosis, reticulating colliquation, and diphtheroid, fibrinoid, colloid, hyaline, parenchymatous, ballooning, or reticulating degeneration. Be that as it may, the type of degeneration in certain virus diseases is somewhat different from that ordinarily observed, inasmuch as the "reticulum" in the vesicles is formed by the

* Cited by White and Robey.

remains of swollen degenerated cells that have lost their nuclei and from which the major portion of the cytoplasm has disappeared (Plate 26, Figs. 1–8).

To investigate the relation of degeneration to proliferation in tissues infected with vaccine virus or smallpox virus, one should observe the phenomena as they occur in the cornea of rabbits. Within a short time after the cornea is inoculated, 3 to 6 hours, changes are seen in the immediate vicinity of the point infected; the epithelial cells are larger and stain less intensely than usual, mitotic figures and amitotic giant cells begin to appear. Within 6 to 24 hours, vaccine bodies are frequently found in affected cells. Small, yet macroscopic nodules are observed on the surface of the cornea 24 to 48 hours after inoculation. Examination of these nodules reveals, in addition to a hypertrophy of individual cells, a definite increase in the number of cells as compared with the findings in control areas. At this time, 48 hours after inoculation, evidences of degeneration and inflammation appear. Guarnieri, in 1892, von Wasielewski, in 1901, and Paul, in 1916, graphically recorded many of the changes just described (Plate 25, Figs. 1–4). Moreover, von Wasielewski stated that the first influence of vaccine virus is to produce increased nutrition and enlargement of cells. Therefore, as far as vaccinal and variolous infections in cornea of rabbits are concerned, one seems justified in concluding that the pathological changes occur in the following order: (1) stimulation and proliferation, (2) degeneration, (3) inflammation.

At this point it is interesting to note that in sheep-pox, a disease similar to vaccinia or cow-pox, Bosc and others have described remarkable proliferative changes in the skin, lungs, and other organs of the body. The proliferation in the lungs (Plate 25, Fig. 5) is evidenced by minute translucent nodules, "sheep-pox adenomas," which represent alveoli completely filled with peculiar, pale-staining cells with vesicular nuclei. Bosc believed the cytoplasmic inclusions (Plate 22, Fig. 22) observed in sheep-pox to be parasites, and, in view of the fact that hypertrophy and hyperplasia of cells are quite evident in infected tissues, he proposed for the inciting organism the name *bryocyte*, an agent causing cells to proliferate.

Mosaic disease in certain plants, frequently spoken of in Germany as "Pockenkrankheit," resembles in many respects the vesicular eruptions observed in animals. This similarity is particularly notice-

able when fruits are attacked (Plate 27, Figs. 1–8). The pathological picture presented by mosaic is said to be characterized by necrosis and hyperplasia occurring in the order mentioned. In view of the fact, however, that mosaic inclusion bodies are found in living cells, some of which are undergoing division, and in the light of what is known concerning other virus diseases, it is possible that further studies concerning the relation of necrosis to hyperplasia in mosaic conditions may reveal some interesting facts.

In molluscum contagiosum the pathological changes in the epidermal cells are evidenced by hypertrophy and hyperplasia (Macallum) which are followed by degenerative activities (Plate 24, Fig. 5). Somewhat the same course of events is observed in warts and condyloma acuminatum. Contagious epithelioma of fowls, or fowlpox, is a disease with two names, each of which suggests a pathological process different from that indicated by the other. At one time this morbid condition was believed to be neoplastic in nature, but at present the tendency is to consider it closely allied to other pock diseases. In spite of the presence of warty growths that characterize the disease, one should remember that a destruction of tissue with vesicle formation also occurs (Plate 26, Fig. 8).

In lesions of infectious myxomatosis of rabbits first described by Sanarelli, the subepidermal tumor-like masses have attracted most attention. Interesting changes, however, have been observed also in the epidermis where a marked swelling and then a complete dissolution of the cells take place. In this disease it appears that proliferative phenomena predominate in the corium and subcutaneous tissues to such an extent that tumor-like masses are formed, while in the epidermis retrograde changes with vesicle formation prevail (Plate 26, Figs. 1–3).

The virus of vesicular stomatitis injected in the pads of guinea pigs causes rapid destruction of epithelial cells, and within 18 to 24 hours after inoculation, vesicles are already well developed. While at this time many swollen cells are still present in the lesions, mitotic figures, amitotic giant cells, and other evidences of proliferation are either absent or present in small numbers (Plate 26, Fig. 7). I have not had the opportunity of examining lesions earlier than 18 hours after inoculation, consequently I do not know whether an appreciable amount of proliferation precedes so rapid a destruction of cells as that observed in this disease. In the light of what is now known con-

cerning the bacteriophage, it is conceivable, however, that such a proliferation may occur.

The lysis * of bacteria by bacteriophage, as the name applied to the phenomenon suggests, has been considered the most important feature of the action of this agent on microörganisms. D'Herelle, Hadley, and others, however, have spoken of an initial stimulation of bacteria as evidenced by an increase in rate of their multiplication. It has also been observed repeatedly that the size of individual cells increases considerably under the influence of phage. The constancy or the importance of this swelling as a factor in the disappearance of bacteria has not been generally accepted. Moreover, it has been suggested that swollen bacteria are very resistant to dissolution and disappear, if at all, very slowly.

The extent of swelling of individual bacteria, the relative proportion of swollen cells, as well as the actual relation between the swelling and the lysis, are difficult to establish by the usual methods of observation. Consequently, Dr. Bronfenbrenner, with the assistance of Mr. Rosenberger, investigated the matter cinematographically. The following is a brief description of their findings: After a short period of lag, bacilli under the influence of phage began to multiply at a rate noticeably exceeding that of normal organisms photographed under similar conditions. Many cells failed to complete their division and filaments having a length of from 10 to 20 times that of normal bacteria were frequently observed. By the end of the first hour of growth, occasional cells had already begun to swell, and by the end of the third hour the majority of bacteria in the field appeared more or less swollen. The swelling continued slowly until about the fifth hour, when, one by one, the bacteria suddenly and quickly disappeared, leaving little, if any, evidence of their former existence (Plate 28, Figs. 1–3).

Bronfenbrenner has also shown that in stained preparations the cytoplasm of swollen bacteria takes the dye less intensely and less evenly than does the cytoplasm of normal cells, in consequence of which it frequently appears segmented or beaded (Plate 28, Fig. 2). These observations were substantiated by photographs of unstained bacteria made by means of ultra-violet light. The rapid melting

* The description of the pathological picture observed in bacteria undergoing lysis is taken from Bronfenbrenner, Muckenfuss, and Hetler's paper, "The study of intimate mechanism of the lysis of bacteria by bacteriophage," *Am. J. Path.*, 1927, iii, 562.

away of the bacteria recorded cinematographically together with observations on the appearance of swollen bacteria in stained and unstained preparations probably indicates that the cytoplasm of bacteria under the influence of phage is liquefied within the cells prior to the disappearance of their membranes.

From the observations recorded it seems that in lesions produced by the majority of viruses, phenomena related to stimulation, proliferation, and degeneration of cells, as well as those connected with inflammation, occur. In some cases, all of the phenomena do not appear, because under the existing conditions it is impossible for certain of them to take place. For instance, in diseases that attack nerve cells, as in rabies, no proliferation of the involved cells has been described. In such diseases the first response of the affected cells is probably an alteration in their metabolism or a necrobiosis. Nor does inflammation in the form of cellular exudate always occur, either because certain tissues are involved, *e. g.*, the epidermis in molluscum contagiosum, or because certain hosts, *e. g.*, plants and bacteria, cannot respond to injury in this manner. Finally, in diseases in which all the phenomena occur, one frequently experiences difficulty in determining the order of their occurrence. In some diseases, however, and under certain conditions in others, the pathological changes apparently take place in the following order: (1) hypertrophy and hyperplasia,* (2) degeneration or necrobiosis, and (3) inflammation in the form of cellular or other exudation.

Relation of Proliferation, Degeneration, and Inflammation to the Formation of Inclusion Bodies

Inasmuch as typical inclusion bodies are frequently observed in cells before any evidences of inflammation in the immediate neighborhood are discernible, it appears that these structures are not directly related to the products of inflammation, *e. g.*, engulfed leucocytes.

Although the question is still open as to whether the incitants of many virus diseases are structurally related to their significant inclusions (enveloped by them), and in spite of the contention of certain workers that inclusions represent parasites, it seems most likely

* Some workers may contend that these changes are the first responses to many kinds of injury and constitute evidences of degeneration. Be that as it may, in any event they are evidences of stimulation and proliferation.

that at least the major portion of the constituents of these peculiar bodies are in some manner related to the phenomena of proliferation and degeneration of host cells. Furthermore, in the majority of instances they appear to be more closely connected with hypertrophy and hyperplasia of cells than with retrograde changes. In rabies, a disease in which little or no proliferation of affected cells occurs, degenerative processes or altered cell metabolism may be responsible for the formation of inclusions. In morbid conditions, however, where proliferation precedes, or at least goes hand in hand with degeneration, and where inclusions are found in actively growing and dividing cells (Plate 22, Figs. 1 and 3; Plate 23, Figs. 1 and 8; Plate 24, Fig. 7) it seems not unlikely that the cellular activity induced by the viruses may lead to the formation of the inclusions either through the overproduction or the modification of some substance or substances normally present in cells, or in consequence of the retention of material ordinarily excreted. These views receive further support from observations concerning the size of cells affected by viruses. In certain instances their diameter increases from 7 to 400 microns (Plate 24, Figs. 1–4). Such an enhancement of size forces one to admit that the changes giving rise to it cannot result from degeneration alone and can take place only in living cells with a fairly active metabolism.

According to these views, inclusion bodies constitute the visible manifestations of a series of activities taking place in living and frequently in growing cells under the stimulating and degrading influences of certain viruses. In most instances the structural relation of the incitants to their specific inclusions is an open question. The distinctive differences observed in inclusions may be dependent upon the species of host, the type of cell and its portion affected, and the nature of the stimulus in the form of virus or its activity. It is true that proliferation and degeneration of cells are observed in some virus diseases and also in diseases other than those caused by viruses without the occurrence of typical inclusions in affected cells. The nature of the stimulus which determines the type and extent of changes taking place in these diseases probably accounts for the absence of inclusions under these conditions.

Summary

There are diseases caused by certain peculiar incitants, viruses, that produce in their hosts pathological changes not entirely unlike those found in other diseases, yet sufficiently different from them in regard to phenomena related to proliferation and degeneration to warrant placing such agents in a group by themselves. If proliferative phenomena predominate, pathological conditions such as warts, molluscum contagiosum, and tumors result. If destructive or retrograde changes prevail, diseases such as varicella, vesicular stomatitis, and lysis of bacteria are the consequence.

Cells affected by many of the diseases in the group exhibit intracellular changes sufficiently characteristic to be spoken of as inclusion bodies. These structures are probably closely related to the proliferative and degenerative phenomena induced in cells by the action of viruses.

The views expressed in the present paper concerning the pathological conditions observed in virus disease are consistent with living or with lifeless incitants multiplying either intra- or extracellularly. The pathological changes, however, as well as other phenomena, emphasize the fact that in virus diseases an intimate type of parasitism exists.

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DESCRIPTION OF PLATES

PLATE 22

FIGS. 1 and 2. Epithelial cells affected by the virus of contagious epithelioma; the pink-staining masses in the cytoplasm represent Bollinger bodies. Fig. 1 also shows the result of amitotic division of the nucleus. Zenker's fixative; eosin and methylene blue stain. × 1700.

FIGS. 3 and 4. Cells, probably endothelial leucocytes, in the interstitial tissue of a rabbit's testicle affected by Virus III. The inclusions are represented by the pink intranuclear masses. Fig. 3 represents an amitotic giant cell. Zenker's fixative; eosin and methylene blue stain. × 1700.

FIGS. 5–7. Human epithelial cells injured by the virus of varicella. The pink masses in the nuclei are the characteristic inclusions. Fig. 7 represents an amitotic giant cell. Zenker's fixative; eosin and methylene blue stain. × 1700.

FIGS. 8–11. These figures represent a, b, e, and g respectively of Abb. 8 in Lauda and Luger's article on herpes simplex, *Ergbn. inn. Med. u. Kinderheilk.*, 1926, xxx, 377, and show the initial stages of oxychromatic nuclear degeneration.

FIGS. 12 and 13. These figures represent a and b respectively of Abb. 9 in Lauda and Luger's article on herpes simplex (see above), and show oxychromatic nuclear degeneration fully developed.

FIGS. 14 and 15. These figures represent 2 cells of Abb. 10 in Lauda and Luger's article on herpes simplex (see above), and show the end stages of oxychromatic nuclear degeneration.

FIGS. 16 and 17. Vaccine bodies — Guarnieri bodies — in the cytoplasm of corneal epithelial cells of a rabbit, 48 hours post inoculation. Zenker's fixative; eosin and methylene blue stain. × 1700.

FIG. 18. Represents one cell from Abb. 18 of Lipschütz's article on the herpes group of diseases, *Arch. Dermatol. u. Syph., Orig.*, 1921, cxxxvi, 428. The cell, corneal epithelial cell of a rabbit inoculated with herpes simplex virus, shows an acidophilic nuclear inclusion. Sublimate alcohol fixation; hämalaun eosin stain; Zeiss 1/12 im. and oc. 4.

FIG. 19. Represents an amitotic giant cell in human genital herpes. From Abb. 10 in Lipschütz's article (see above). Sublimate alcohol fixation; Giemsa's stain; Zeiss 1/12 im. and oc. 4.

FIG. 20. Represents a human epithelial cell affected by the virus of herpes zoster. From Abb. 1 in Lipschütz's article (see above). The characteristic change is the acidophilic mass in the nucleus. Sublimate alcohol fixation; Giemsa's stain; Zeiss im. and oc. 4.

FIG. 21. Reproduction of a cell from Fig. 5, Plate V, of Negri's article on rabies, *Z. Hyg., u. Infectionskrankh.*, 1903, xliii, 507, and shows a cell from Ammon's horn of a dog that died of rabies 15 days post infection. In the cytoplasm is a typical Negri body. Zenker's fixative; Mann's stain; Zeiss 2 mm. and oc. 6.

FIG. 22. Reproduction of a cell from Fig. 15, Plate II, of Bosc's article on sheep-pox, *Centr. Bakt., 1. Abt., Orig.*, 1903, xxxiv, 413, 517, 666. It is an epithelial cell with a cytoplasmic inclusion and vacuolated nucleus. Flemming's fixative; magenta phenol picro-indigo-carmine stain; Zeiss 1/12 im.

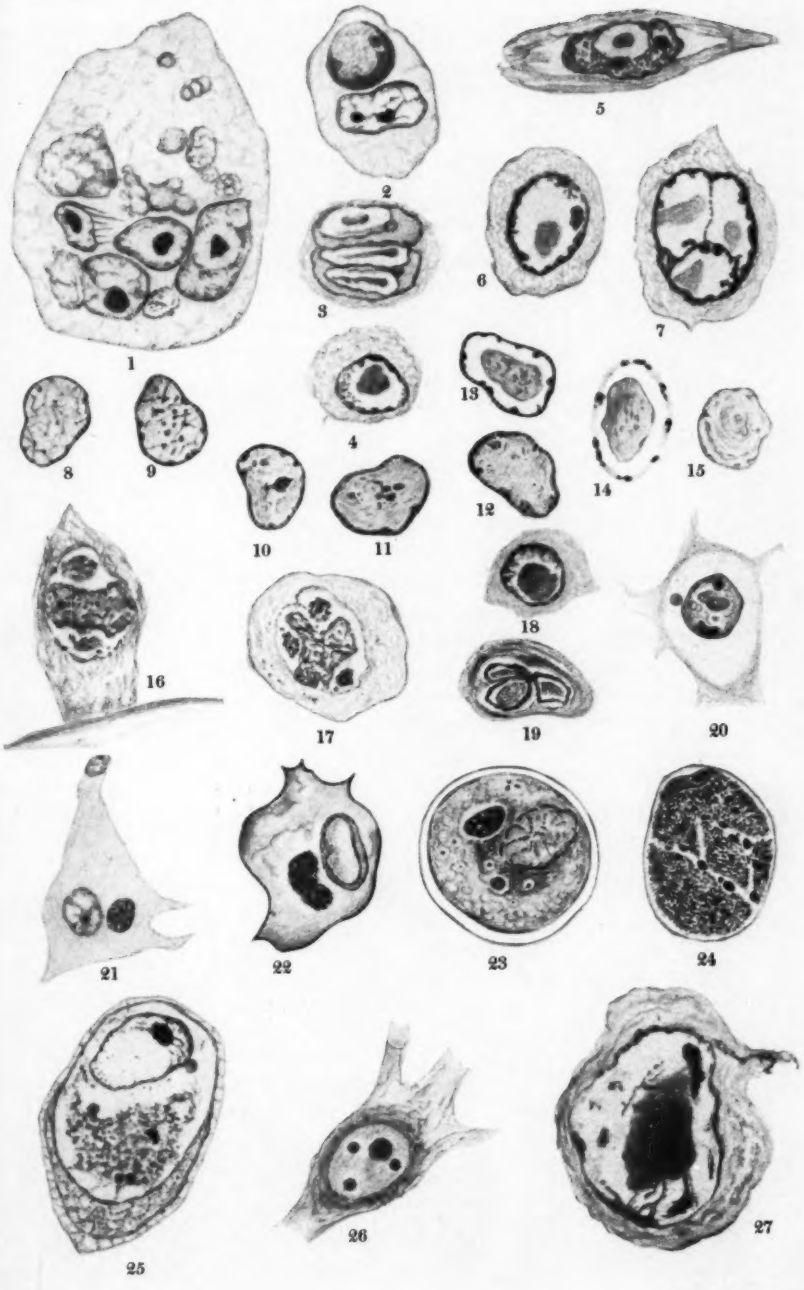
FIG. 23. Represents Fig. 5 (slightly modified) of Plate XXVIII illustrating Weissenberg's article on lymphocystic disease of fish, von Prowazek's Handbuch der pathogenen Protozoen, 1921, Leif. 9, 1344. The cell, 27 microns in diameter, shows several cytoplasmic inclusions. Acetic alcohol fixation; hematoxylin and Biondi stain. × 1025.

FIG. 24. Reproduction from Plate XVI illustrating Lipschütz's article on molluscum contagiosum, *Arch. Dermatol. u. Syph.*, 1911, cvii, 387, represents an epithelial cell with its nucleus compressed and pushed to the periphery and with its cytoplasm practically replaced by a so-called molluscum body. Sublimate alcohol fixation; Giemsa's stain; Leitz im. 1/12 and oc. 8.

FIG. 25. Epithelial cell of rabbit affected by the virus of infectious myxomatosis. The cell is swollen, the nucleus is vacuolated, and the cytoplasm contains a large granular pink-staining mass in the midst of which are several blue coccoid bodies. Zenker's fixative; eosin and methylene blue stain. × 1700.

FIG. 26. Reproduction of Fig. 5, Plate II, illustrating Joest's chapter on Borna disease in Kolle and von Wassermann's Handbuch der pathogenen Mikroorganismen, Jena, Fischer, 2nd ed., 1913, vi, 251. Ganglion cell from Ammon's horn showing pink nuclear inclusions. Lentz's stain; Zeiss im. 1/12 and oc. 3.

FIG. 27. Reproduction of one cell from Fig. 1, Plate 33, illustrating Cole and Kuttner's article on salivary gland virus of guinea pigs in *J. Exp. Med.*, 1926, xliv, 855. It is a swollen duct cell with eosin-staining nuclear inclusion from a submaxillary gland of a full grown pig. Zenker's fixative; eosin and methylene blue stain. × 1700.



Rivers

Filterable Viruses

Plate 23

Fig. 1. Tobacco mosaic. "Young palisade cell containing striate material radiating from the nucleus and a crescent-shaped body (A) adjacent to nucleus." (After Rawlins and Johnson, *Am. J. Bot.*, 1925, xii, 19.)

Fig. 2. "Leaf cell containing mitotic figure, crescent-shaped body (A), and striate material." (After Rawlins and Johnson.)

Fig. 3. "Cell of a short glandular trichome containing a body (A) of the same type shown in Fig. 4." (After Rawlins and Johnson.)

Fig. 4. "Cell of a short glandular trichome showing striate material and vacuolate body (A) containing a dark-staining granule." (After Rawlins and Johnson.)

Fig. 5. Intracellular body (marked X) in rosette-diseased wheat. (After McKinney, Eckerson, and Webb, *J. Agric. Res.*, 1923, xxvi, 605.)

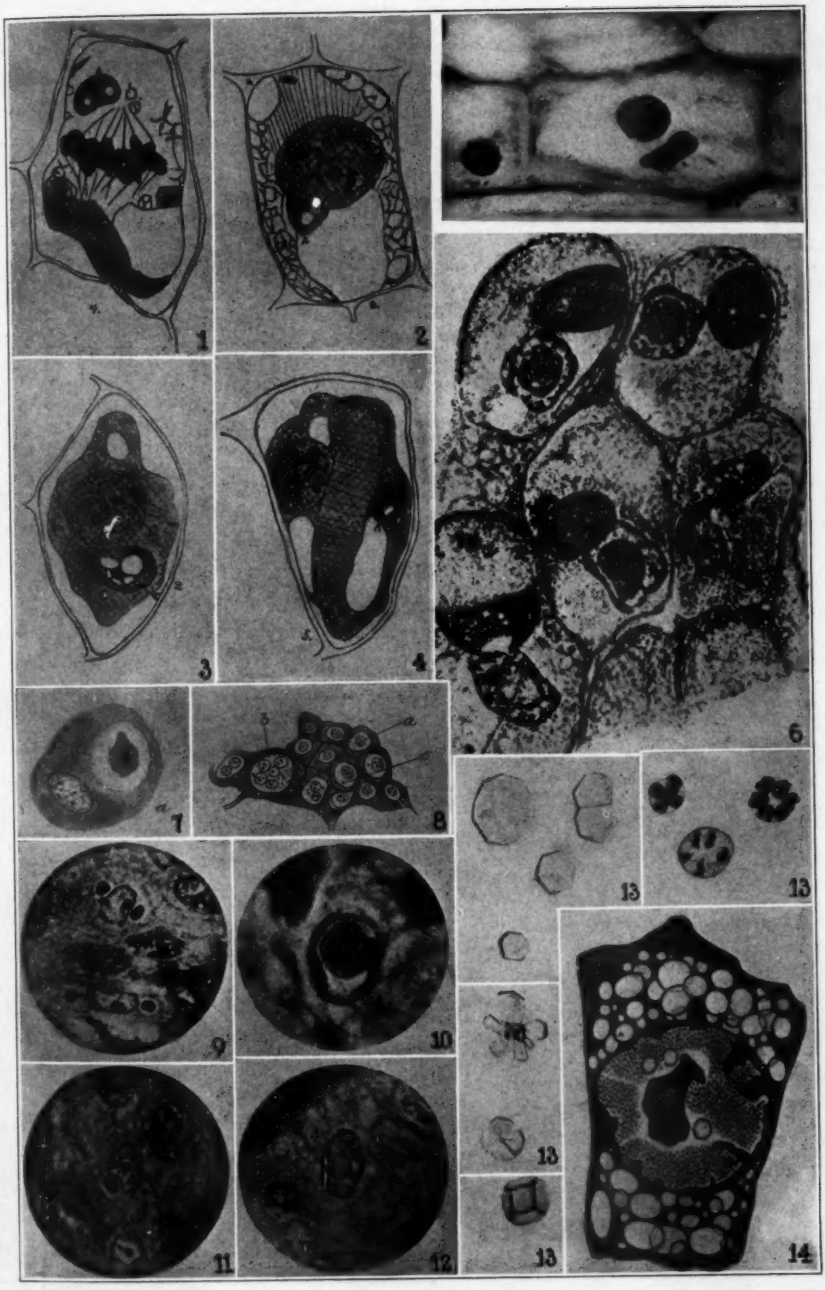
Fig. 6. Shows nuclear and cytoplasmic inclusions in paravaccinia. Reproduction of Fig. 12, Plate III, in Lipschütz's article on paravaccinia, *Arch. Dermatol. u. Syph., Orig.*, 1919–20, cxxvii, 193.

Fig. 7. Reproduction from Guarnieri's article on vaccinia in 1892 showing a vaccine body in the cytoplasm.

Fig. 8. Reproduction from Weigert's article on smallpox in 1874 showing amitotic giant cells and bodies in cytoplasm of affected cells similar to the ones later described by Guarnieri.

Figs. 9–12. Cells showing nuclear and cytoplasmic inclusions in smallpox. (After Councilman, Magrath, and Brinckerhoff, *J. Med. Res.*, 1904, xi, 12.)

Figs. 13 and 14. Polyhedral bodies in jaundice of silkworms. (After von Prowazek, *Centr. Bakt., 1. Abt., Orig.*, 1912–13, lxvii, 268.)



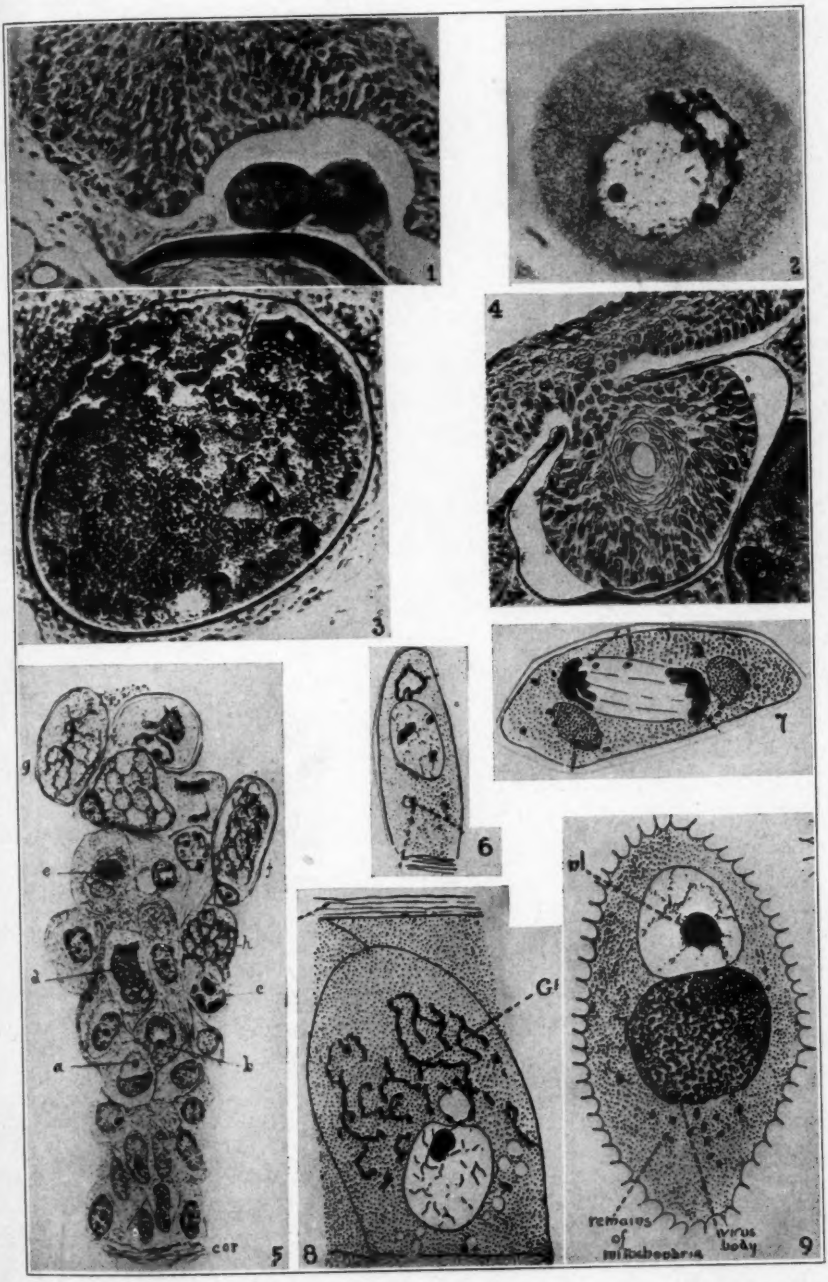
Rivers Filterable Viruses

PLATE 24

FIGS. 1–4. Illustrations by Joseph (1918) showing the remarkable increase in size of cells affected by the virus of lymphocystic disease of fish. In Fig. 1, there are three small dark cells showing the early effect of the virus, and two large ones exhibiting the picture presented at a later stage. Fig. 3 presents a cell going to pieces. Fig. 4 shows new cells filling the space left by one degenerated cell. Fig. 1 = × 250; Fig. 2 = × 650; Fig. 3 = × 200; Fig. 4 = × 200.

FIG. 5. Reproduction from Macallum's (1892) article showing the changes that take place in epidermal cells under the influence of molluscum contagiosum virus.

FIGS. 6–9. Reproductions from Ludford and Findlay's article (1926) on fowl-pox. Fig. 6 shows small vacuole with minute adherent granules. Fig. 7 represents a cell with a mitotic figure and two virus bodies. Fig. 8 shows reversal of Golgi apparatus. Fig. 9 presents a well developed virus body in a greatly swollen cell.



Rivers

Filterable Viruses

Plate 25

Fig. 1. Reproduction of illustration by Guarnieri (1892) showing hyperplasia and hypertrophy of corneal cells with vaccine bodies caused by the action of vaccine virus.

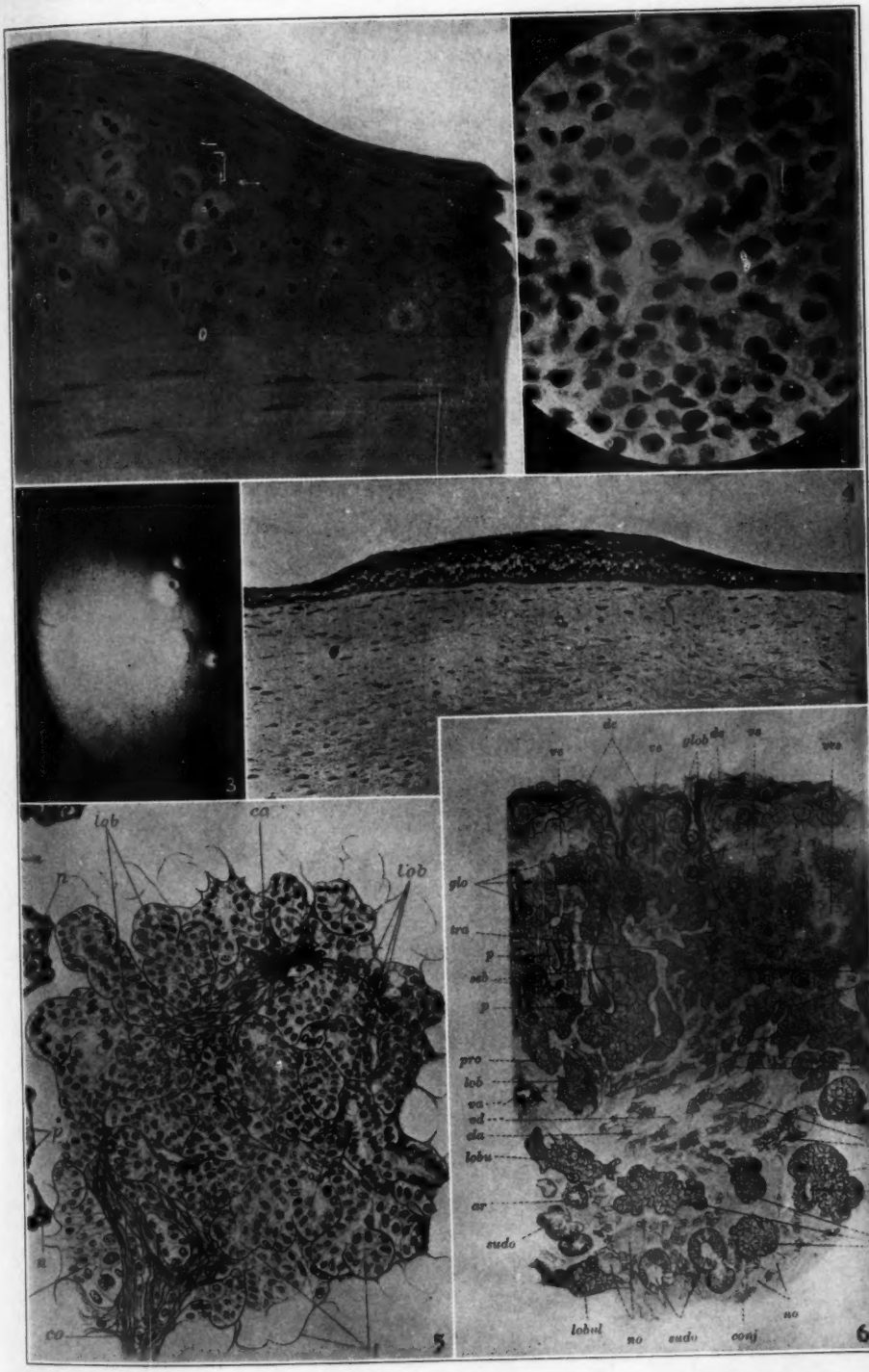
Fig. 2. Reproduction of illustration by von Wasielewski (1901) showing hyperplasia and hypertrophy of corneal cells with amitotic giant-cell formation caused by the action of vaccine virus.

Fig. 3. Illustration by Paul (1916) showing the effect of smallpox virus on the corneal cells of a rabbit 86 hours after inoculation.

Fig. 4. Illustration by Paul (1916) showing hyperplasia and hypertrophy of corneal cells caused by smallpox virus 48 hours after inoculation. No evidence of inflammation at this time.

Fig. 5. "Sheep-pox adenoma" of lungs. (After Bosc, 1903.)

Fig. 6. Hyperplasia and hypertrophy of cells in the skin caused by the virus of sheep-pox. (After Bosc, 1903.)



Rivers

Filterable Viruses

PLATE 26

FIG. 1. Epidermal cells covering a myxomatous mass in the corium (virus myxomatosum, Sanarelli). Some of the epithelial cells are swollen and contain cytoplasmic inclusions. × 375.

FIG. 2. Epidermal cells undergoing dissolution. Compare with Fig. 1. × 125.

FIG. 3. Complete dissolution of epidermal cells. Compare with Figs. 1 and 2. × 125.

FIG. 4. "Degenerated cells showing vacuolation of protoplasm and shrivelling of nuclei." (After Councilman, Magrath, and Brinckerhoff, 1904.)

FIG. 5. "Reticular degeneration of epithelial cell." (After Councilman, Magrath, and Brinckerhoff.)

FIG. 6. "Early vesicle showing formation of epithelial reticulum." (After Councilman, Magrath, and Brinckerhoff.)

FIG. 7. Vesicle in pad of guinea pig 18 hours after inoculation with virus of vesicular stomatitis. Reticulum formed by degenerated cells. × 125.

FIG. 8. Vesicle formation that frequently occurs in contagious epithelioma or fowl-pox. × 125.

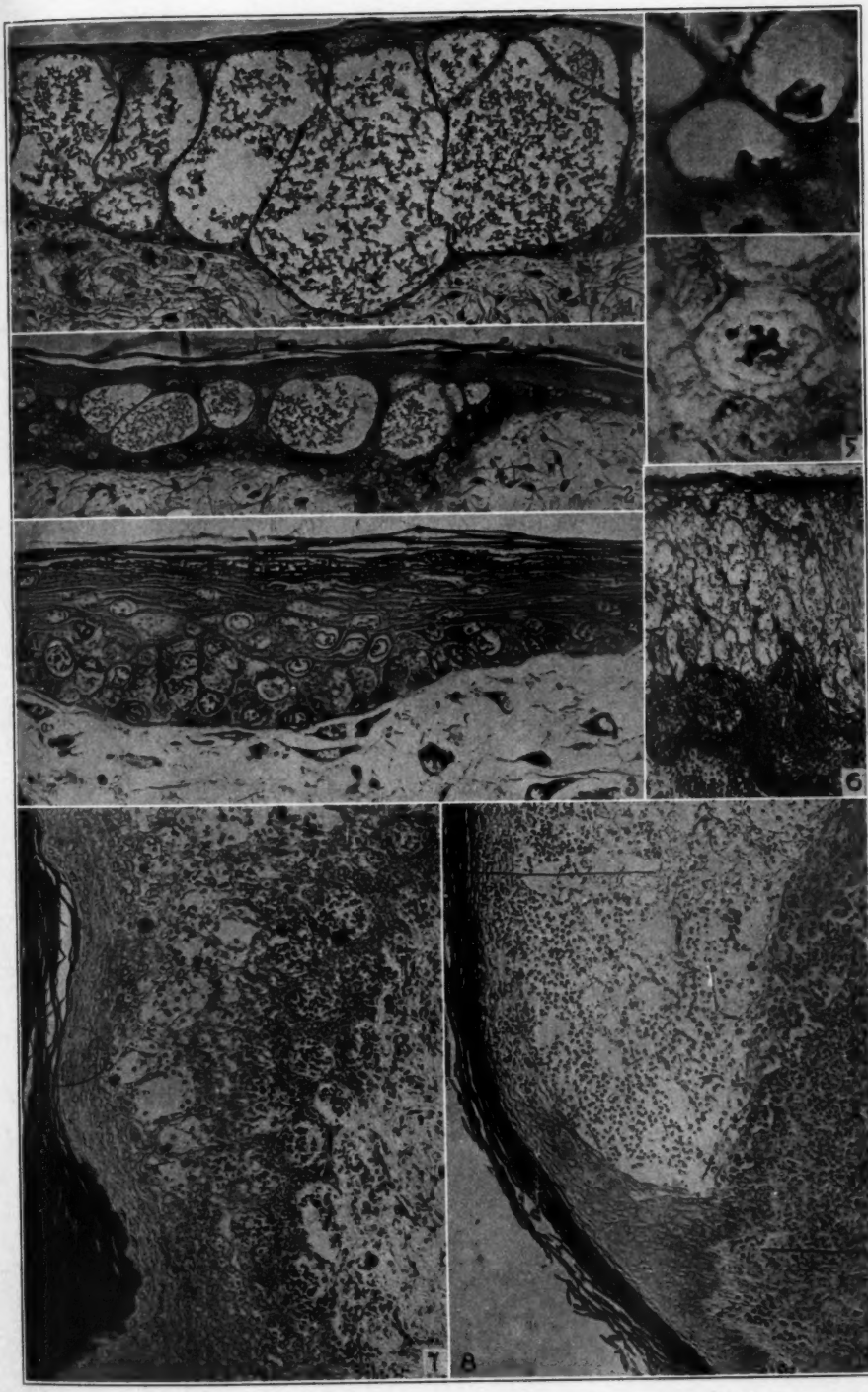

5
6
7
8

PLATE 27

FIG. 1. "Crown tissue from rosette-diseased plant" (wheat). "Note the cells containing intracellular bodies in addition to the nuclei and also the granular nature of the cells which have become necrotic." (After McKinney, Eckerson, and Webb, *J. Agric. Res.*, 1923, xxvi, 605.)

FIG. 2. "Crown tissue from healthy plant." Compare with Fig. 1. (After McKinney, Eckerson, and Webb.)

FIG. 3. "Section of an early stage of a fruit blister showing the brown necrosis of the epidermal cells and the hyperplasia of the subepidermal cells." (After Gardner, *J. Agric. Res.*, 1925, xxx, 871.)

FIG. 4. "Section of a pericarp lesion resulting from necrosis and collapse of an area of subepidermal cells and hypertrophy of the cells immediately beneath the necrotic area." (After Gardner.)

FIG. 5. "Surface blisters on a mosaic tomato." (After Gardner.)

FIG. 6. Mosaic leaf of a gourd. (After Doolittle, *U. S. Dept. Agric. Bull., No. 879*, 1920.)

FIG. 7. "Mosaic fruit of pumpkin, showing large dark-green swellings on a yellow background." (After Doolittle.)

FIG. 8. Mosaic disease of Summer Crookneck squash. (After Doolittle.)

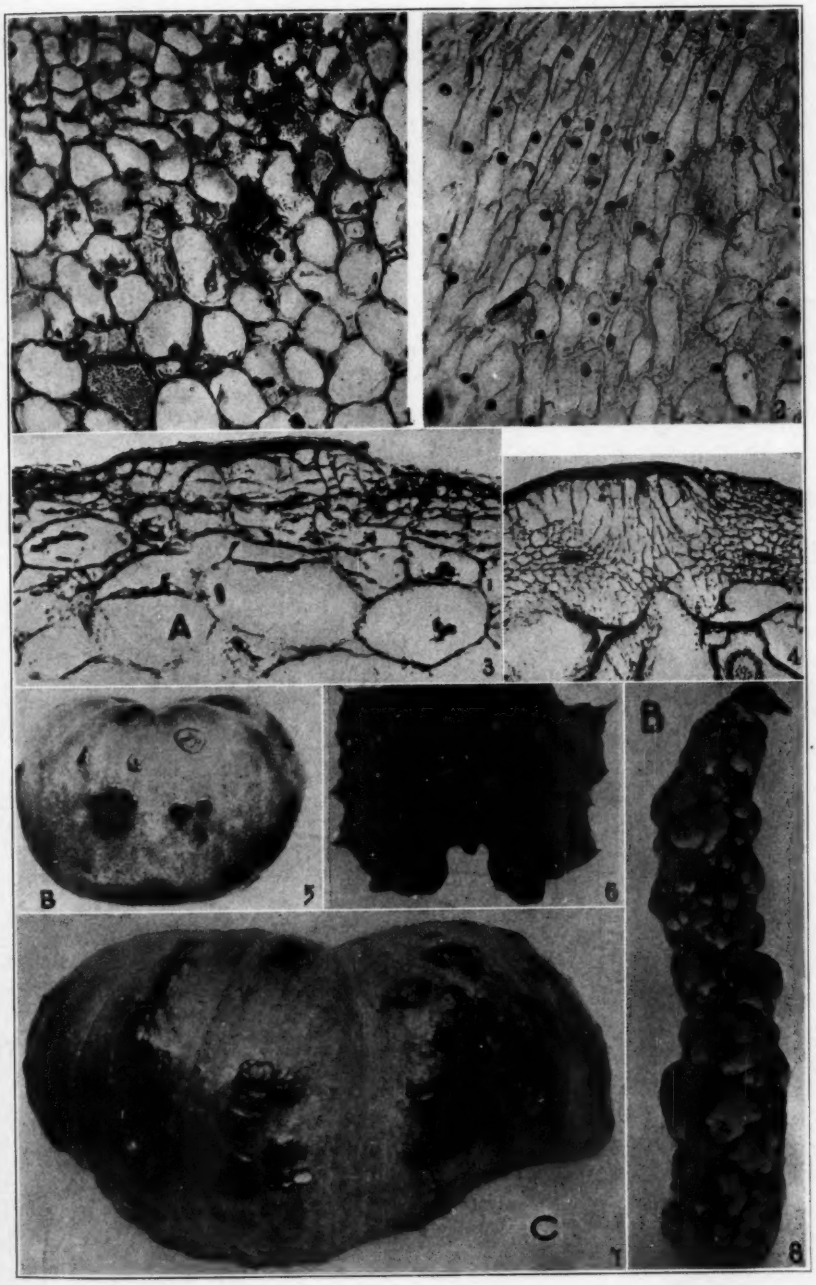

A
B
C
B

PLATE 28

FIGS. 1 and 3. Fig. 3 is an enlargement of Fig. 1. At the left side of Fig. 3 are normal bacteria. The large organisms in the center of the picture show the swelling induced by the action of bacteriophage. Pictures supplied by J. J. Bronfenbrenner.

FIG. 2. Stained preparation of bacteria undergoing lysis. The protoplasm is beaded and segmented. Picture supplied by J. J. Bronfenbrenner.

Rivers Filterable Viruses

A STUDY OF THE HISTOPATHOLOGY OF THE SO-CALLED ADENOSARCOMA OF SWINE *

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To the student of comparative pathology, the occasionally encountered neoplasm of the kidney and sublumbar region of swine offers an interesting field of investigation. This tumor has been called by a great variety of names: adenoma sarcomatode, adenomyosarcoma, sarcocarcinoma, sarco-adenoma, rhabdomyo-adenosarcoma and adenosarcoma, the last being the term most commonly used by pathologists in veterinary medicine and by meat inspectors. The variety of terms used to designate this tumor attests to the lack of agreement as to its true nature; however, little has been contributed to the literature of comparative pathology during the last two decades that might assist in clarifying a conception of this interesting neoplasm.

Kinsley [1] gives a brief description of the growth but fails to justify the use of the term adenosarcoma, while Day,[2] who was the first to describe these tumors of swine, gives an excellent description of the tumor and also presents a formidable array of evidence to substantiate his use of the term adenosarcoma. Most of the standard textbooks on pathology and veterinary medicine fail even to mention this growth, which suggests its comparative rarity, as well as the lack of information concerning it. After reviewing Kinsley's and Day's descriptions of this neoplasm it is evident that they use the term adenosarcoma because of certain resemblances which they believe it has to the embryonal adenosarcoma of the child's kidney. In fact Day embraces Herzog and Lewis' [3] view explanatory of the histogenesis of the embryonal adenosarcoma of the child's kidney as probable in the case of such tumors in swine.

With such a paucity of literature it is not surprising that the term adenosarcoma has been taken for granted in referring indiscriminately to all tumors encountered in the kidney of swine. I believe that this designation places undue emphasis on the connective

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tissue constituents with a consequent disregard of the importance of the epithelial elements which commonly predominate.

Report of Cases

During five years I have collected eleven specimens of the tumor under consideration. All of these were received from packing houses where clinical observations were necessarily limited and as a consequence the following data are somewhat meager.

Case 1. The animal was slaughtered for food and subjected to the usual necropsy examination. One of the kidneys was involved in a large tumorous process. The entire mass was received at my laboratory.

The tumor measured 15 by 25 cm. It had an irregularly oval shape and was gray in color and firm in texture. The surface was smooth and covered by a white tough capsule. Fragments of what appeared to be renal tissue were scattered throughout part of the tumor near its attachment. The mass weighed 2200 gm.

Case 2. A tumorous mass was observed in the body of the right kidney of a two-year-old Duroc sow which had been slaughtered for food. The carcass was otherwise in good condition.

The tumor was a firm, sharply circumscribed mass embedded in the body of the kidney, particularly involving the medullary portion. The growth approached the capsule at the hilum. It measured 4 by 7 cm., and was gray in color and somewhat irregular in shape. The surface was roughened and invested with a capsule and a freshly cut surface showed distinct mottling of the tissue.

Case 3. A two-year-old Poland China hog was slaughtered for food and at necropsy a tumor was discovered in the sublumbar region adjoining and posterior to the kidneys. The general physical condition of the carcass was good; and no other structures were involved.

The mass was spherical and about 25 cm. in diameter. It was flesh-colored and soft. The surface was smooth and covered by a capsule. A cross-section of the mass revealed some irregular spongy areas, as large as 1 cm. in diameter, which contained light brown papillary projections. The tumor seemed quite vascular at the time of its removal from the carcass.

Case 4. The animal was an eight-month-old Duroc male hog, which was slaughtered for food. At necropsy the carcass was found to be in good condition with the exception of a mass lateral and distal to the left kidney.

The tumor was a firm, bean-shaped mass, measuring 38 by 30 cm. and weighing nearly 8 kg. (16.5 pounds). The tumor was gray, rather vascular and the surface was covered with a capsule which could be removed without difficulty. It was also covered with a serous membrane.

Case 5. A year-old male Duroc hog was slaughtered for food and the carcass subjected to the usual necropsy examination. A diffusely attached tumor was observed involving the region of the hilum of the left kidney.

The tumor had an irregular roughened surface and measured about 5 by 5 cm. It was firm in consistence and of a grayish white color with a few dirty yellow areas scattered throughout its substance.

CASE 6. A hog in apparent health was slaughtered for food. During the usual necropsy inspection of the carcass a mass was observed near the surface at the end of one kidney.

The tumor was a grayish oblong structure with a slightly roughened surface surrounded by a very thin capsule. The mass was firm in some places and spongy in others. It measured 12.5 by 6 by 4.5 cm.

CASE 7. A hog was slaughtered for food and a neoplasm was found in the position ordinarily occupied by one of the kidneys although but little renal tissue was present.

The mass measured 20 by 10.5 by 20 cm., was gray, quite firm, and was surrounded by a capsule. It was oval and flattened. A freshly cut surface showed strands of grayish white tissue cutting the tumor into small irregular lobular areas.

CASE 8. A hog had been slaughtered for food after having passed a satisfactory antemortem inspection. When the carcass was eviscerated one kidney was found to possess a number of grayish white areas of varying sizes, most of them slightly raised above the surface (Fig. 1).

The kidney was cut through longitudinally and the grayish tissue was seen in some places extending almost through the cortical portion of the organ. Freshly cut sections of the kidney through the grayish white areas showed them to be diffusely in contact with the substance of the organ, with no attempt at encapsulation. The line of contact of the tumorous tissue with that of the kidney was quite uneven. The tumor was of about the same consistence as the kidney proper.

CASE 9. At antemortem examination there was no manifestation of the tumor which occupied the left sublumbar region of a two-year-old female hog which had been slaughtered for food. The tumor was rather massive and measured 42 by 28 cm. in its greatest dimensions. It weighed 9.5 kg. (about 19 pounds). The renal tissue had practically disappeared and all that remained of the organ was a flattened atrophic layer on the surface at one side of the tumor. The mass was elongated and quite irregular (Fig. 2). When the tumor was cut through, multiple cysts were found which contained foul-smelling fluid. The interior of the tumor was mottled. Cartilage or bone was not demonstrable.

CASE 10. A hog, age and sex not given, was slaughtered for food and a tumor of the left kidney was observed.

The kidney showed a roughened nodular protuberance at one pole which was of a lighter color than the rest of the organ. When the kidney was divided longitudinally it was found that practically the entire interior consisted of abnormal tissue with the renal substance limited to a shell-like structure at the periphery. The tumor was attached firmly in the region of the protuberance while the bulk of the mass was invested with a capsular covering which seemed to be deflected to line the renal cavity in which the tumor rested.

CASE 11. A seven-month-old hog was slaughtered for food. The animal was in fair condition and there was no antemortem evidence of disease. Both kidneys were affected with diffuse lobulated tumors. The right kidney measured 15 by 9.5 cm. and weighed 540 gm. The left kidney measured 20 by 10 cm. and weighed 810 gm. Tumors presented a compact, mottled type of structure with but little kidney tissue remaining (Fig. 3).

Gross Characteristics

Site: These neoplasms may involve the renal tissue, or, in extreme cases in which the renal tissue has been replaced by the tumorous proliferation, they are found in the place ordinarily occupied by this organ and extend backward into the pelvis and forward perhaps to the diaphragm. Occasionally the kidneys are intact and the tumor appears posterior to the kidney in the sublumbar region. The tumors are usually unilateral but bilateral involvement may be encountered (Case 11).

Shape: The shape is variable, the growths for the most part being oval but often presenting an irregular contour with deep lobulations.

Size: In bulk the tumors vary from a small focus just visible to the eye to huge masses weighing several kilograms. Day reports one that weighed nearly 27 kg. and another that weighed 12 kg. The largest in my series measured 42 by 28 cm. and weighed 9.5 kg. (19 pounds). In a personal communication received recently from Dr. E. W. Barthold, Austin, Minnesota, he mentioned that several years ago he had encountered a specimen which weighed more than 31.5 kg. (66 pounds). This must approach the maximal size for these tumors.

Color and consistence: The majority of these tumors are grayish white, and they are rather firm. Some show dirty yellow areas scattered promiscuously throughout; those that are soft often are flesh-colored.

Capsule: In the larger specimens a capsular covering is always present which is never particularly adherent and is not difficult to remove. The extremely large tumors may have a serous covering which is a deflection of the peritoneal membrane carried along with the tumor as it grows and encroaches on the abdominal space.

Retrogressive changes: Cystic cavities are frequently a feature of the larger growths. The contents of the spaces may be serous fluid of a gelatinous nature. Occasionally spongy areas of variable dimensions are seen in the depths of the tumor. These are usually darker than the surrounding tissue and probably represent phases of necrosis. In the larger specimens there is sometimes solution of tissue with resultant foul-smelling purulent liquid occupying the necrosed areas. Freshly cut surfaces frequently present a distinctly mottled

appearance due to strands of grayish white fibrous tissue which cut the tumor into small irregular lobular areas (Fig. 3).

Pathologic Histology

A microscopic study of the eleven tumors shows that they fall into two rather distinct groups. Seven tumors (Cases 1, 2, 6, 7, 8, 9, 10) are purely epithelial in nature with the connective tissue elements serving as nothing more than stroma for the epithelium of the parenchyma, without the slightest indication of malignant behavior. The other four tumors (Cases 3, 4, 5 and 11) should be considered as mixed tumors with a vigorously growing epithelial portion making up the bulk of the growths and minor areas of embryonic fibroblastic tissue exhibiting certain characteristics which simulate those of fibrosarcoma. To the second group the term embryonal adenosarcoma might be properly applied, while those first described would be correctly considered embryonal adenocarcinoma. Briefly, the morphologic features of the two groups follow:

Adenocarcinoma: With few exceptions the make-up of this type of growth is quite cellular, the parenchyma being irregularly interrupted by broad septums of connective tissue which often carry a few blood vessels. From these larger septums smaller strands are frequently given off which pass into the epithelial areas to become lost in the complex maze of their ramifications. The type of cell may be either the low cuboidal to low columnar variety or a peculiar elongated spindle-like cell which takes an intense nuclear stain. The cytoplasm of the latter variety may be slightly granular and the nucleus, which resembles an oat kernel in contour, usually occupies a considerable portion of the cellular bulk. Owing to the intense basic-staining qualities of the nucleus it is usually impossible to study anything more than the contour and size of this cell. Occasionally a tumor is encountered in which both types of epithelial cell are represented, some portions showing the low columnar or cuboidal variety, while in other areas the spindle type of cell with the deeply staining nucleus, shaped like an oat kernel, constitutes the parenchyma (Case 9, Figs. 4 and 6).

In areas in which the spindle type of cell is present one often finds a peculiar tuft-like arrangement of the parenchyma, which suggests a renal corpuscle (Figs. 4 and 5). Capillary vessels are absent, the

structure consisting only of convolutions of flattened epithelium and fibrous stroma within a cavity which is lined with a continuation of the small flattened cells that constitute the tuft-like portion. Usually the cells of the parenchyma are arranged in an irregular tubular or alveolar fashion with occasional acini and epithelial papillae projecting into the cavities (Fig. 6). In many instances the epithelial cells are not confined to the lining of the tubular or alveolar spaces but in an undifferentiated state are promiscuously disposed in great numbers in the intertubular areas. Mitotic division of the epithelial cells is often seen and eosinophils are occasionally observed in the intertubular or interalveolar areas. In the larger specimens of this tumor, the renal tissue may be entirely replaced by the neoplastic elements, while in the earlier types the tumor cells may be seen invading the surrounding renal substance in a most menacing fashion. This type of tumor is similar in most details to the characteristic adenocarcinoma found elsewhere, except perhaps in the matter of metastasis.

Adenosarcoma: A phenomenon in the growth of adenosarcoma is that the fibroblastic elements in many of the areas ordinarily looked on as stroma exhibit the same malignant neoplastic tendencies as the adenomatous portions described for adenocarcinoma. In certain fields these fibroblastic cells have a distinct embryonic appearance and mitosis can be frequently demonstrated. The epithelial portions are distinctly adenocarcinomatous (Figs. 7 and 8).

In the mixed type of tumor it is not unusual to find epithelial duct-like structures in the midst of fields of young proliferating fibroblasts. With Van Gieson's stain one occasionally finds, in certain portions of the stroma, some strands of tissue with the characteristic light yellow color of smooth muscle. Day and others have described the occurrence of cartilage and bone in certain portions of these tumors but I failed to demonstrate either of these tissues in the material at my disposal.

In *both types* of the tumor, cavities or cysts of variable size may be encountered. Some are microscopic while others measure from one to several centimeters in diameter. These are usually lined with a single layer of cuboidal epithelial cells. The content of these cavities is a homogeneous substance which stains pink with eosin and resembles, in most respects, epithelial hyalin although the color reaction with Van Gieson's stain is not entirely typical for

this material. Areas of hydropic degeneration associated with necrosis of the tumor cells are occasionally encountered. Ample blood supply is insured by the many vessels running in the stroma of connective tissue and by the capillary structures which are sometimes seen in the midst of the cellular parenchyma.

Metastasis: While *both types* of the tumor are capable of exhibiting a destructive, advancing type of growth in so far as the renal substance is concerned, metastasis to the regional lymph nodes and to distal parts of the body, while possible, is seldom observed. Day described metastasis in two cases, the lung being involved in one and the sublumbar lymph nodes in the other.

In none of my cases was metastasis demonstrable, although it must be admitted that a thorough search for secondary foci was not made at the time the carcasses were eviscerated because of the lack of time and the failure of the examiner to appreciate the importance of this phase of the necropsy.

Wollstein [4] contends that metastasis from these tumors in the child is hematogeneous and that the regional lymph nodes are seldom invaded. Packard and Blumberg [5] report that in the child metastasis may appear in the vertebrae. This observation in swine has not been reported.

Symptoms

Compared to the closely related neoplasms of man which are considered to be particularly malignant, the variety in swine seems benign. In man they produce symptoms of a grave organic disturbance and usually terminate fatally in the first few years of life. In children metastasis to lung or liver is common. In swine, on the other hand, there has been no instance, so far as I can determine, in which these tumors have been diagnosed or even suspected during life. That these tumors in swine are not detected during life may be accounted for at least in part by the horizontal posture of this animal. This position causes the abdomen to be more or less pendulous and as a result large renal tumors may press downward unnoticed in a part of the body which is normally of imposing dimensions. In the child the upright posture and the anatomic configuration of the abdomen render the detection of such tumors fairly easy even before they attain large proportions. Even in those animals in which, at necropsy, large tumors were found, symptoms of suffi-

cient magnitude to command attention were absent. These large tumors must interfere to some extent with the digestive process by extension into the space ordinarily necessary for the proper housing of the abdominal viscera. No doubt the animal experiences sensations of discomfort from large abdominal neoplasms, such as man experiences. If the tumor is unilateral and involves the kidney proper there must be gradual compensatory hypertrophy of the unaffected organ. In the rare cases of bilateral renal involvement, grave consequences will certainly follow the gradual encroachment of the neoplasm on the renal substance.

The fact that most swine are slaughtered at a comparatively early age, undoubtedly accounts for the failure to observe the symptoms which might be expected from tumors of such menacing possibilities. Perhaps the tendency of the tumor to become encapsulated assists in preventing its spread. If metastasis occurs and the disease is permitted to run its course, the usual effects of malignant disease can be predicted.

Histogenesis

In studying these tumors one cannot avoid the conclusion that they bear a close relationship to the embryonal renal tumors occasionally observed in children, usually referred to as embryonal adenosarcoma, although there is not complete agreement as to the use of this term. The histogenesis of these growths offers an interesting field of speculation and many theories have been offered in explanation. While the hypotheses of various authors differ in many respects, there seems to be agreement that these tumors develop as a result of some congenital mishap. The exact nature of the anomaly is not yet determined, although there is suggestive evidence that it is the result of some fetal inclusion, a fetal rest or a fetal displacement.

Wollstein in a recent paper presents a logical explanation of the origin of these tumors. She states: "As to the origin of these renal neoplasms it seems probable that they all come from the same type of embryonal cell, but that they come from the cell at different stages of its differentiation and consequently of its potency. The more embryonic and less differentiated the cells from which the tumor originates, the more complicated will its structure be because of the multipotency of the undifferentiated mesodermal cells. Thus

for the rhabdomyo-adenosarcomas it seems simple to presuppose a parent cell with an inherent ability to give rise to striated muscle, supporting tissues and kidney elements, since these are all mesodermal in origin and closely approximated in the young embryo, and the inclusion of such early cells within the developing kidney is possible. This was Wilms'[6] view. It seems the only way to account for the striated muscle tumors, for striated muscle cannot develop from unstriated muscle, and so metaplasia gives no adequate explanation of these growths. The presence of cartilage and bone in the mixed tumors is also explained. For the less complex tumors two possible origins suggest themselves. Either the parent cells are more differentiated, of later origin, and so have the potentiality only of the renal and supporting tissue elements or the cells of the early renal blastema are adequate for the development of the tumors containing only kidney elements like tubules, connective tissue and unstriated muscle. Again, growths with more embryonal epithelial elements may take their origin from an earlier cell of the renal blastema than that which gives rise to the more adenomatous forms, when the cells have reached the stage of differentiation of renal tubular epithelium. Since these neoplasms are not all alike, but only closely related, there seems no special reason why a single origin must be involved for them as a group."

This is, in the main, the opinion of Nicholson[7] who believed it "reasonable to conclude that such tumors have originated in areas of undifferentiated kidney substance which are known to occur." Fraser[8] attributed the origin of the adenomatous tissue to a group of erratic nephrogenetic cells and of the sarcomatous tissue to metaplasia of the adenomatous elements. Neither of these authors believed that the Wolffian body was responsible in any way for the origin of the growths.

Certainly it is not necessary to use the Wolffian body to explain tumors of this type which occur, as the majority do, within the substance of the kidney proper. An occasional tumor of this variety is found entirely separate from the kidney and it would seem logical to assume that the Wolffian body was at least indirectly implicated in its origin and that the anomaly probably results from failure of certain portions of the mesonephros to be utilized in the production of permanent renal tissue. This failure results in so-called fetal rests from which the tumor develops. Considering the early age at

which these tumors become manifest, it is hardly likely that the forerunner of tumor tissue remains long inert. On the contrary, there probably never is cessation of growth. It proceeds without the directing influences of normally developing tissues and becomes an erratic, ungoverned mass of cells with all the characteristics and possibilities of true tumor.

The occurrence of the embryonic neoplasms apart from the kidney must be rare since I have found only two cases (in man) in the literature reviewed. Hedren's case quoted by Wollstein presented bilateral involvement in which the kidneys, while compressed by the adjacent tumors, were intact and not invaded by neoplastic cells. Hood and Albert [9] also described an embryonal tumor in a child whose kidney was free from neoplastic involvement although flattened by the proximity of the growth. This case was interesting in that evidence of slight metastasis was observed in the adjacent intestinal loops. The kidney in contact with the tumor was described as showing marked fetal lobulation and slight hydronephrosis. In two of my cases (Cases 3 and 4) the tumor was in the sublumbar region and did not involve the kidney. Both were of the mixed type and could be correctly designated as embryonal adenosarcoma. The occasional observation of these tumors posterior to the kidney suggests the possibility that they occur anterior to this organ as well. The tuft-like arrangement of the flattened cells in certain of the adenocarcinomas of this group (Fig. 4) suggests further that these tumors arise from nephrogenetic cells at different stages of differentiation. This structure has a general resemblance to a renal corpuscle (Fig. 5). On close examination the cells in this arrangement appear similar in many respects to the flattened epithelial cells constituting Bowman's capsule of the normal kidney.

It would appear that cells, which were destined in the ordinary course of events, to invest the glomerulus, had undergone some differentiation in preparation for their normal purpose, then had suddenly lost the directing influence of normal nephrogenic tissue, and as a consequence of this loss of restraint had continued to proliferate in a haphazard, aimless manner. Occasionally they are found lining narrow crescent-shaped cavities with the inner layer arranged in folds or convolutions suggesting that the true function of the cells is to line the glomerular space and cover the glomerular structure. However, since glomerular elements are missing, the cells intended

to invest them proliferate and practically fill the cavity with multiple folds of flattened epithelial cells.

Discussion

The occurrence of tumors of this type in swine further emphasizes the fact that neoplasms fail to respect species. Practically every kind of tumor which may affect man has been observed in the lower animals. It is a little peculiar, however, that these embryonal growths should be limited among the lower mammals to swine, for there is no evidence that they have been observed in other species. Why swine alone should be susceptible to these tumors and other mammals escape offers an interesting problem for the embryologist. *

The early age at which the majority of these tumors are found, especially in children, suggests a congenital inception. When these renal tumors occur, children are far from the so-called cancer age, indicating that the growth, perhaps in many cases, is well under way at birth. In this connection it is interesting to note that Kastner, quoted by Wollstein, found a spindle-cell sarcoma of the kidney in a seven-months fetus. Nicholson mentioned a case of adenosarcoma of the left kidney in a woman aged forty. In this instance growth must have been very slow which is in opposition to the behavior of the majority of these tumors in man.

The life of swine compared to that of man is short and as a consequence it is difficult to determine whether there is an age in swine when tumors are more prone to occur. Available data would indicate that the tumors in question are the most commonly encountered neoplasms in swine. That these tumors are seldom observed suggests further the relative immunity of swine to neoplasms which arise except as a result of congenital anomaly. In a previous communication[10] dealing with the incidence of tumors in the lower animals, it was shown that of a total of 132 tumors which were collected for study in a period of more than four years, nine species were repre-

* Since this article was written I have reviewed the papers of Bell and Henrici,[12] Scott[13] and Polson[14] on embryonal tumors of the kidney of the rabbit. Also that of Baird[15] who described an embryonal epithelial tumor from the right kidney of a chicken which he concluded was the "remnant of an early stage renal anlage." Through the courtesy of Dr. Frank P. Mathews, La Fayette, Indiana, I have also had the privilege of studying several tumors of the kidney of the chicken; these are unquestionably embryonic in nature and similar in most respects to those of the kidneys of swine.

sented. Fifteen of these tumors occurred in swine and of these, nine (60 per cent) were tumors of an embryonal nephrogenic type. The other six were; myxoma one, melanoblastoma three, lymphosarcoma two. While this is perhaps a rather small series from which to draw conclusions, the data at least suggest the relative freedom of swine from the ordinary epithelioblastomas which are so common in the cow and horse.

As to the nomenclature of these newgrowths, it has been the custom of most observers to consider all of them, both clinically and histologically, as adenosarcoma. I prefer the term embryonal nephroma. So far as gross characteristics go there can be no special objection to either of these terms which have become fixed by long usage, but microscopically it is possible and desirable to separate these tumors into two and possibly three groups, although for the cases described in this paper two groups would suffice.

While the groups unquestionably have many features in common, I fail to see any good reason why all should be labeled the same histologically. There is justification for the use of a common term by which they may be designated grossly since structural detail cannot be determined macroscopically, but there is no reason why the pathologist should use a single term since differences in structure are apparent to anyone who has the opportunity to study these growths histologically. While in every case the microscopic picture is embryonal in character, there is often considerable difference in the dominant element. This difference also obtains in the tumors of this type observed in children. Wollstein spoke of this feature in her recent paper and it was also mentioned by Hinman and Kutzman.[11]

In tumors in which the epithelial elements constitute the major portion of the structure with the connective tissue elements clearly acting as supporting stroma, as in glandular epithelioma, it would seem proper to use the term embryonal adenocarcinoma. To designate these as adenosarcoma is without good reason, since cells with sarcomatous tendencies are lacking. Those with a mixed type of architecture, possessing in addition to epithelial nests and tubular structures a variable amount of embryonic connective tissue elements such as fibroblasts, bone, cartilage, smooth and striated muscle, could be designated embryonal adenosarcoma indicating the adenomatous structure associated with tissues possessing sarco-

matous tendencies. I have not observed an example of the third group in the relatively few specimens I have studied. Wollstein, however, described a typical fibrosarcoma from the kidney of a six-month-old male infant. The structure was devoid of any epithelial elements and the connective tissue stroma was scant. The growth recurred soon after operation and the disease terminated fatally three and a half months after the initial symptoms, which attests to the rapidity of growth possible in this type of embryonal neoplasm. While a pure embryonal fibrosarcoma from the kidney of swine has not been described it would be presuming too much to say that it does not occur. A proper examination of all tumors of the renal area would undoubtedly reveal this type which of course must constitute a minority of these peculiar tumors.

Summary and Conclusions

A review of the literature indicates that these tumors have received but scant attention from the standpoint of comparative pathology.

Eleven embryonal tumors of swine are described, nine of which had their origin in the kidney and two posterior to this organ in the sublumbar region.

A comparison of the specimens from swine with the embryonal renal tumors of the child indicates that the process is comparable in the two species and that sufficient similarity exists to justify the assumption that there is a common histogenesis.

The two tumors in the sublumbar region possibly owed their origin to the failure of certain portions of the mesonephros to be used in the development of the permanent kidney.

The embryonal renal tumors of swine, like those of the child, constitute a heterogeneous group. Grossly it is difficult if not impossible to separate them but histologically two, or perhaps three, types exist. They fall under the following headings: embryonal adenocarcinoma, embryonal adenosarcoma, and embryonal fibrosarcoma. A pure embryonal fibrosarcoma of the kidney of swine has not as yet been described.

Available data indicate that this group of tumors constitute the most common neoplasm of swine, a species which seems to possess a relative lack of susceptibility to tumorous proliferations.

Unlike the closely related group of tumors of the child, those of swine do not appear to embarrass the health. Perhaps this is due in part to the relatively early age at which most swine are slaughtered.

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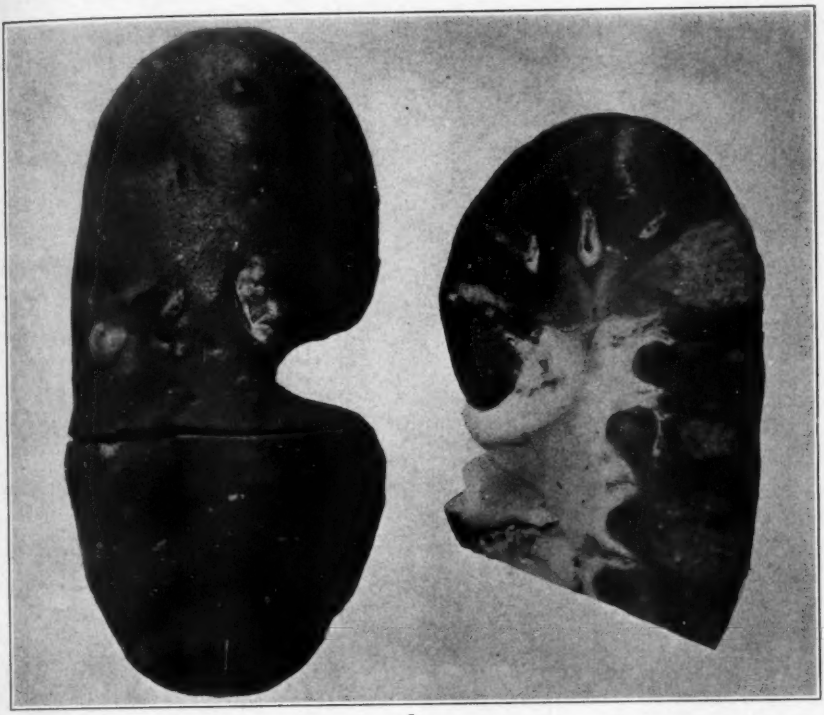
DESCRIPTION OF PLATES

Plate 29

FIG. 1. (Case 8.) Extensive renal involvement by the tumor which presents multiple foci. The distribution of the tumorous tissue is clearly shown in the cortical zone and over the surface of the organ. This specimen represents an earlier stage of the disease than is usually seen.

FIG. 2. (Case 9.) Gross appearance of tumor. Remaining renal tissue so small in amount as to be practically indiscernible.



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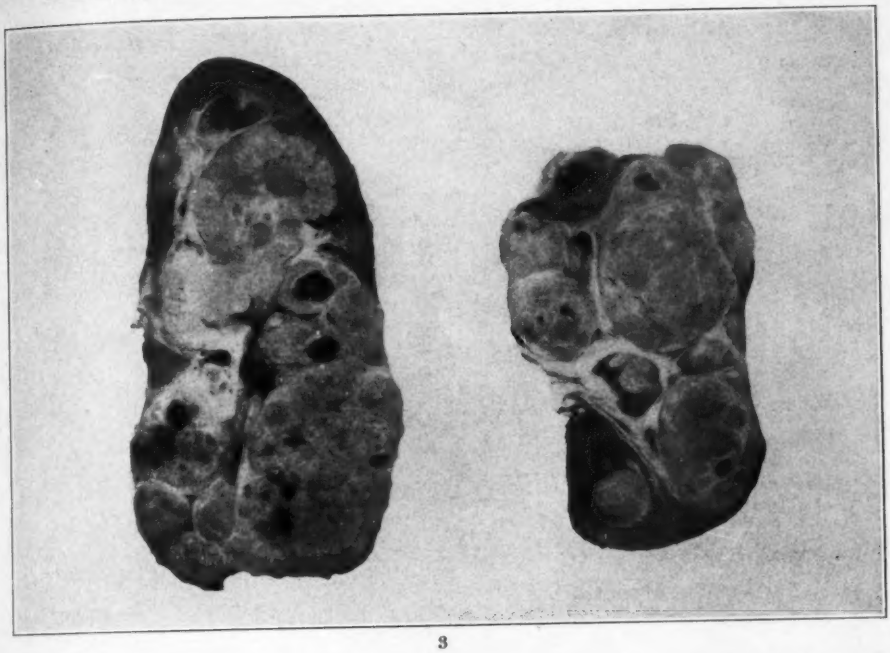
Feldman Adenosarcoma of Swine

PLATE 30

FIG. 3. (Case 11.) Bilateral embryonal adenosarcoma. Longitudinal sections of left and right kidneys which show the small amount of kidney tissue present and the mottled lobular character of the tumors.

FIG. 4. (Case 9.) Tuft-like arrangement of the flattened epithelial cells. The architecture of this embryonal adenocarcinoma has some general resemblance to a renal corpuscle. Compare with Fig. 5. × 400.



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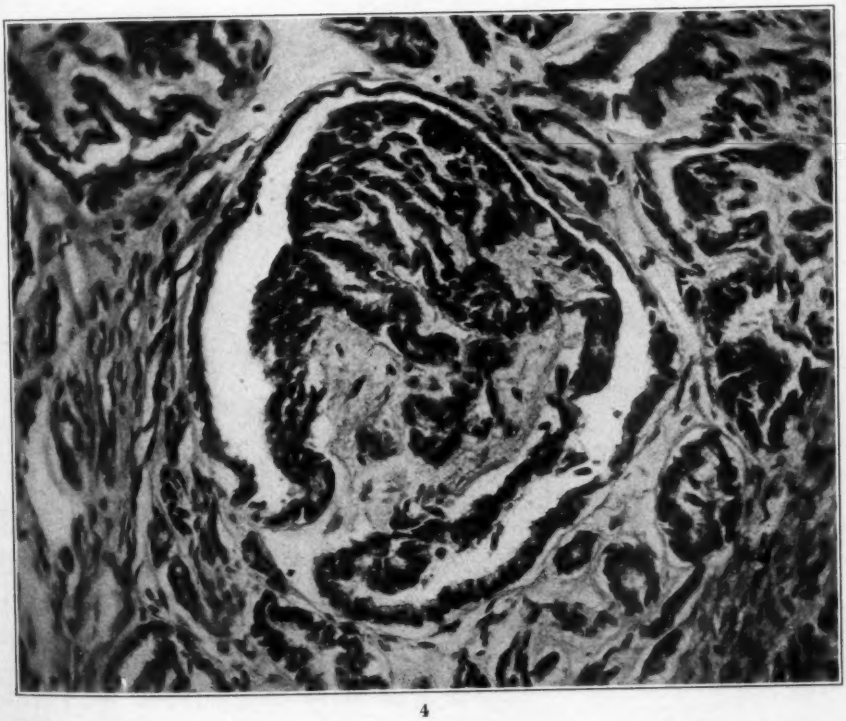

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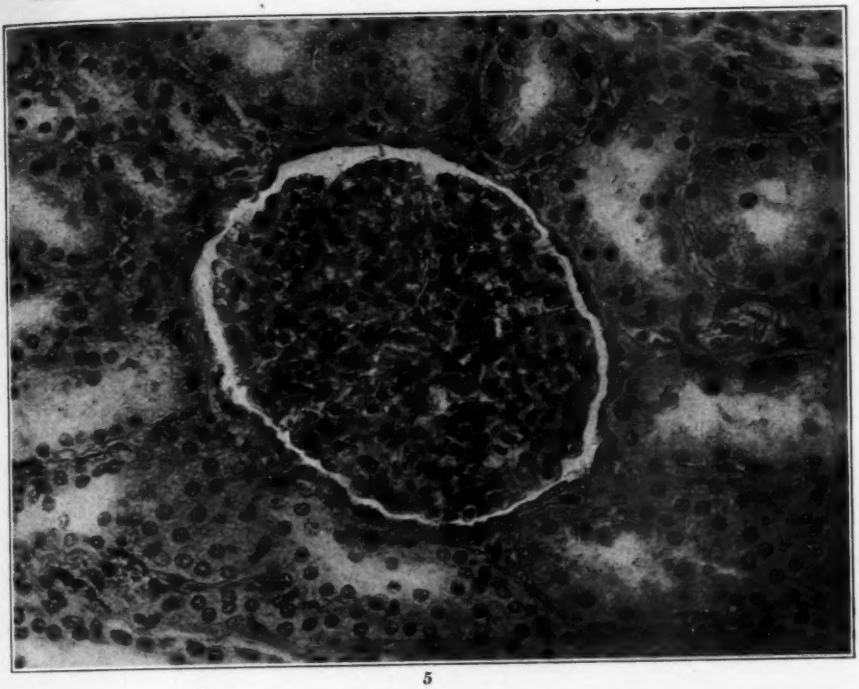
Feldman

Adenosarcoma of Swine

Plate 31

FIG. 5. A portion of a normal kidney of the swine showing one renal corpuscle. × 400.

FIG. 6. (Case 9.) Highly cellular tubular type of architecture of an embryonal adenocarcinoma. × 575.



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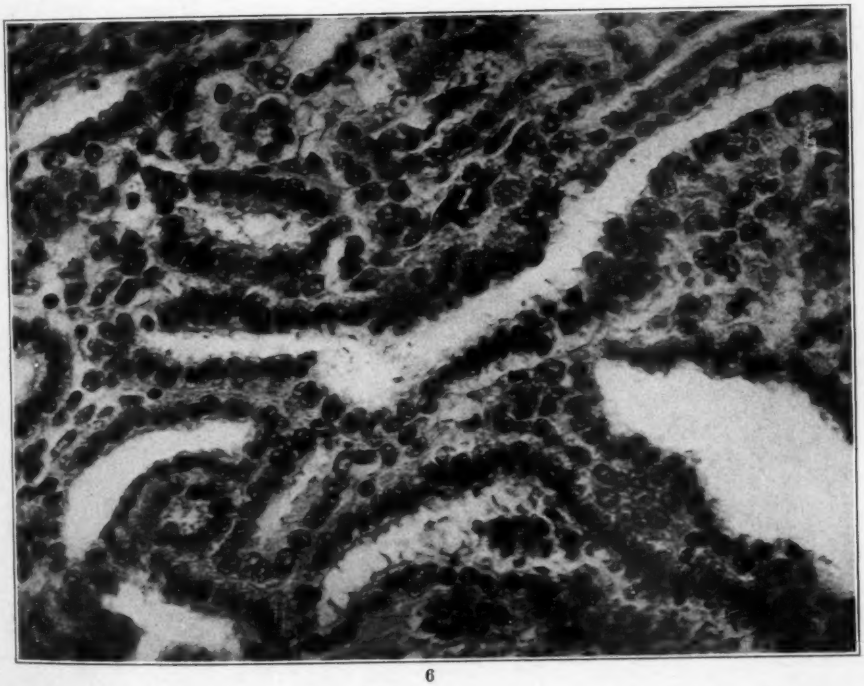

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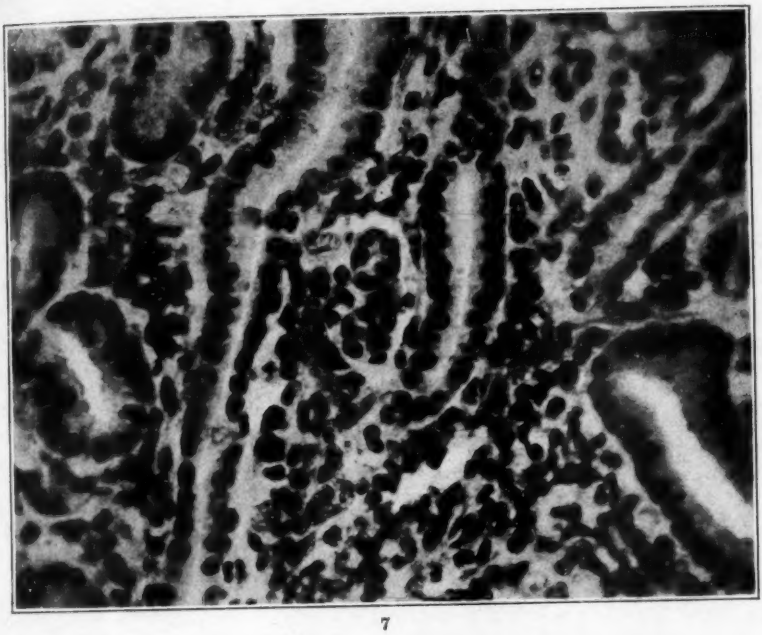
Feldman Adenosarcoma of Swine

PLATE 32

FIG. 7. (Case 4.) The gradual transition of the epithelial cells lining the tubular-like structure from cuboidal to columnar is shown. An epithelial portion of an embryonal adenosarcoma. × 600.

FIG. 8. (Case 5.) An epithelial area of an embryonal adenosarcoma showing the tubules lined by a single layer of cells several of which show mitotic division. × 600.



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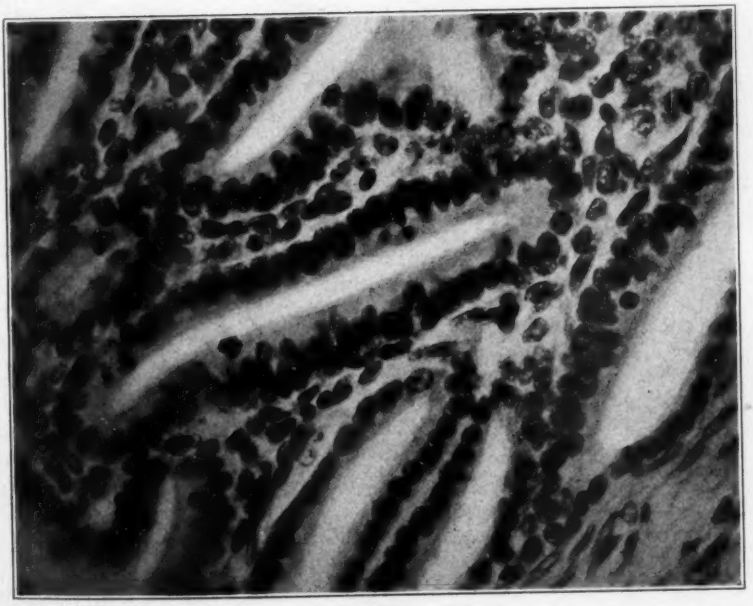

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Feldman Adenosarcoma of Swine

LEIOMYOSARCOMA OF THE SPLEEN IN A BOVINE *

WILLIAM H. FELDMAN, D.V.M., M.Sc.

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The relative infrequency of splenic tumors in human beings has been commented on by many writers. Certainly there is little on the subject in the available literature. Smith and Rusk [1] state, "Malignant neoplasms arising primarily in the spleen are found so infrequently as to be considered pathologic curiosities and so have been of little interest to the clinician." In a recent comprehensive paper on splenic neoplasms by Krumbhaar [2] the following appears: "Splenic neoplasms are of especial value in the study of cancer as well as of the spleen on account of their comparative rarity and the relation that this may have to the supposed rôle of the spleen in combating tumors and infections." Krumbhaar reports 6500 necropsies which yielded a total of 930 primary and 1234 secondary tumors of which only forty occurred in the spleen. Of the forty splenic tumors six were primary and thirty-four were secondary. Leiomyoblastoma of the spleen was not observed. Smith and Rusk noted reports of 104 splenic tumors and reported two original cases which they considered endotheliomas. They did not observe a case of leiomyoblastoma, and considered lymphosarcoma the most common tumor of the spleen.

According to Smith and Rusk benign tumors of the spleen are not infrequently found both in man and in lower animals. If one excepts the so-called lymphomatous tumors of the dog's spleen which are fairly common, but which may not be true tumors, the statement of these writers relative to splenic tumors in animals is not substantiated by the literature or by my own observation. In fact literature on comparative pathology contains practically nothing pertaining to splenic neoplasms in lower animals. Hutyra and Marek [3] mention only the melanosarcoma of the horse's spleen, and Fox [4] failed to observe any splenic tumors in a series of ninety-four neoplasms from more than 5000 captive wild mammals and birds. In an observation of approximately 250 tumors representing nine dif-

* Received for publication January 9, 1928.

ferent species of the lower animals over a period of ten years I have encountered primary splenic tumor only once; this case is reported here. Secondary tumors of the spleen in dogs and chickens affected with lymphocytoma are not uncommon.

I have failed to note in the literature any case in man or animal in which leiomyoblastoma originated in the spleen. MacCallum [5] believes that the walls of the uterus are the usual site of origin of leiomyoblastoma, and that the kidney, bladder and walls of the stomach and intestines are rarely involved. He does not mention its occurrence in the spleen. Mallory [6] says that aside from lymphoblastoma other primary tumors of the spleen are rare. He also believes that the uterus is the most common site for the development of leiomyoblastoma, and that occasionally a tumor of this variety arises in the kidney and subcutaneous tissues. Its presence in the spleen is not considered. Ewing [7] states that primary sarcoma of the spleen is rare; he does not mention the occurrence of leiomyoblastoma in this organ.

Leiomyoblastoma in the lower animals is more common than the literature would indicate. In Fox's case the tumor occurred in the uterine cornua and fimbria of an elephant. Malvicini [8] reported a fibroleiomyoma in the uterus of a lioness and Frohner [9] reported three cases in which the tumors affected the small intestine of a horse. Coca [10] reported a diffuse leiomyoma of the stomach which resulted in rupture of the organ. My own collection contains leiomyoma from the oviduct of chickens, from the uterus of a cow and several from the wall of the cecum of dogs.

Report of Case

The animal was an old cow. The previous history was not available. At necropsy a large tumor was found in the spleen and numerous nodules of various sizes were distributed over practically the entire liver. No other demonstrable lesions were found although careful search was made of all internal organs.

Gross description of the spleen: The tumor which was situated near one end of the spleen was firmly embedded in the substance of the splenic tissue. The mass was oval and was raised above the free surface of the spleen. The serosa was not ruptured but invested the growth in a taut glistening covering. In sharp contrast

to the color of the splenic tissue the tumor was yellowish white. It measured 9 cm. by 4 cm. The neoplastic tissue was firm and was separated from the splenic substance by a rather sharp line of demarcation (Fig. 1). A small secondary nodule was present in the splenic pulp just outside the major portion of the growth.

Pathologic histology: The splenic tumor was invested with a flattened capsular structure which consisted of a mixture of fibrous and smooth muscle tissue suggesting that it was a continuation of the splenic capsule. The neoplastic elements were simple, consisting of strands of tissue the various collections of which were laid down at different angles (Fig. 2). It was impossible to discern the cellular outlines although the nuclei were sharply defined. In cross-section the nuclei were circular, while longitudinally they were slender and elongated, the average being about six times longer than their diameter. The nuclei were fairly constant in width throughout their length, with abruptly formed, rounded ends predominating. Many of the nuclei near one extremity revealed what appeared to be a nucleolus. An abundance of finely granular chromatin material was present. Mitosis was common (Fig. 3). The intercellular material was fine, fibrillar and wavy, running parallel to the longitudinal axis of the nuclei. Sections stained by Van Gieson's method showed a few wavy fibers that gave the color reaction of fibrous connective tissue. These ran in the same direction as the other smaller fibrils which constituted most of the intercellular substance. This stain showed that, aside from the capsule, there was little connective tissue present. Many areas showed evidence of considerable necrosis which was mostly of a coagulative variety. The tumor possessed a generous blood supply, the vessels ranging from minute channels to small arteries. The blood supply was irregularly distributed and the structure of the vessels was characterized by simplicity; it consisted of nothing more than a layer of endothelium resting on the surrounding neoplastic elements.

Gross description of the liver: The liver was slightly enlarged and its external appearance was striking. The surface was irregularly roughened by a large number of nodular swellings of variable size measuring from minute foci to masses 7 cm. in diameter. They were whitish yellow and rather firm. Cross-sections through different areas of the organ showed the nodules to be distributed promiscuously throughout (Fig. 7). The hepatic tissue appeared com-

pressed, due to the pressure exerted by the tumor, and most of the larger vessels were compressed from the same cause.

Pathologic histology: Sections of the liver were obtained from several areas, and while there were minor differences the structure for the most part was a duplication of the splenic tumor. The hepatic foci were perhaps more cellular than in the splenic tumor and mitosis was more abundant (Fig. 4). The capsule remained intact and immediately beneath it there were compressed rows of atrophied hepatic cells running parallel to the capsule. Neoplastic tissue was promiscuously infiltrating the hepatic substance in every direction. Many new bile ducts were present in areas quite devoid of other hepatic elements. A few small areas of necrosis were present but in no sense was this change as extensive as in the splenic material. The blood supply was similar in character to that of the splenic growth. By Van Gieson's stain the same type of reaction was observed as in the sections of the spleen. The fibrous connective tissue fibrils were few in number, wavy in character, and larger than the other fibrils which gave the Van Gieson reaction for smooth muscle elements (Fig. 5). In some parts of the liver the tumor tissue showed signs of regression, the cellular constituents being atrophic and densely arranged. Practically no normal hepatic cells were found in any of the many sections examined from this organ; all were atrophic and flattened by the pressure exerted by the neoplastic tissue which pushed into the hepatic substance from many directions (Fig. 6).

Discussion

According to Smith and Rusk, the tumors capable of arising from the tissue elements of the spleen fall into three main groups: (1) fibroma and fibrosarcoma from the capsular and trabecular framework; (2) lymphoma and lymphosarcoma from the lymphoid elements, and (3) those from vascular or sinus endothelium. In view of the histology of this organ the possibility of a smooth muscle tumor should be considered. Smooth muscle cells are present in the vessel walls and in the capsule and septums in association with fibroblasts. My observations agree with those of Jordan,[11] who states that the splenic capsule of the ox has an abundance of smooth muscle. The trabeculae also possess a large amount.

The foregoing facts together with the morphologic features of the

tumor reported here would seem sufficient to justify the designation of leiomyosarcoma. The reasons for assuming that the tumor was primary in the spleen with the liver affected metastatically appear convincing. The splenic tumor was limited in its development to one focus while the hepatic tumors were extensively distributed throughout the organ, as might be expected in a case of malignant primary splenic tumor which metastasized by way of the blood stream, the tumor cells reaching the liver by way of the splenic and portal veins. If the tumor cells in the portal circulation were abundant and commonly present they would, as a consequence, find lodgment quite generally throughout this organ.

Anatomically the structure of the liver makes it practically impossible for metastasis to occur from one lobe to another. Consequently, when all lobes of the liver contain numerous foci the point of primary origin of the tumor must be sought elsewhere. In the absence of lesions apart from the spleen in this case, the spleen must be considered responsible for the histogenesis of the tumor.

The scarcity of splenic lymphatics emphasizes the importance of the blood stream as a vehicle for the transportation of malignant cells both from and to the spleen. Primary splenic malignancy would appear metastatically first in the liver, where it would be likely to be confined unless the cells as a consequence of vigorous proliferation broke through the lobular capillaries and were carried by the hepatic veins to the posterior vena cava, in which case the lungs would also receive them.

The scirrhous change described as being present in certain of the sections of the tumor from the liver is mentioned by Mallory as one of the retrogressions likely to overtake a leiomyoblastoma. This is due, in Mallory's opinion, to interference with nutrition, as a consequence of which the more delicate smooth muscle cells perish while the more resistant fibroblasts persist although often atrophic.

Summary

From a review of the literature pertaining to splenic neoplasms in man and the lower animals it is apparent that such tumors are seldom seen. In the primary splenic tumors reviewed, fibroblastoma, lymphoblastoma and endothelioma were the varieties encountered. Although the histologic elements for such a tumor are

present in the spleen no case of primary leiomyoblastoma of this organ has been previously described so far as can be determined from the literature reviewed. A case of primary leiomyosarcoma of the spleen of a cow with extensive multiple metastasis to the liver is reported.

Note: Owing to the tissues being fixed in formalin it was impossible to apply specific stains for the demonstration of myoglia fibrils.

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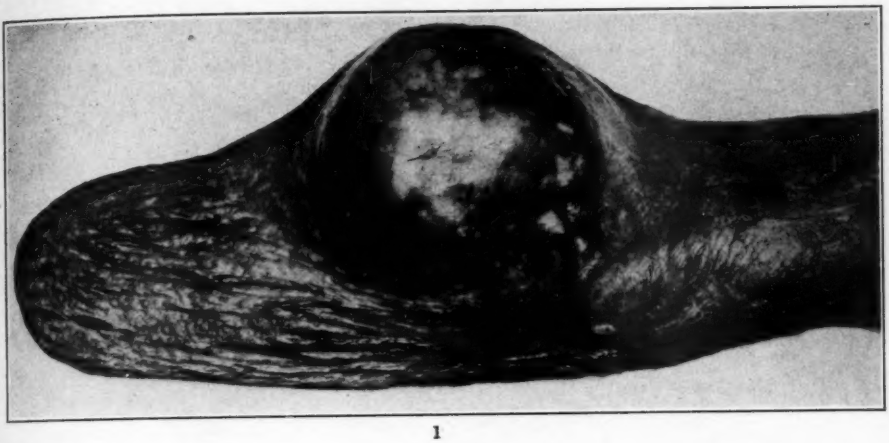
DESCRIPTION OF PLATES

PLATE 33

FIG. 1. Primary leiomyosarcoma of the spleen showing the position of the tumor and its relative size.

FIG. 2. Leiomyosarcoma of the spleen. Simplicity of structure is shown. × 150.



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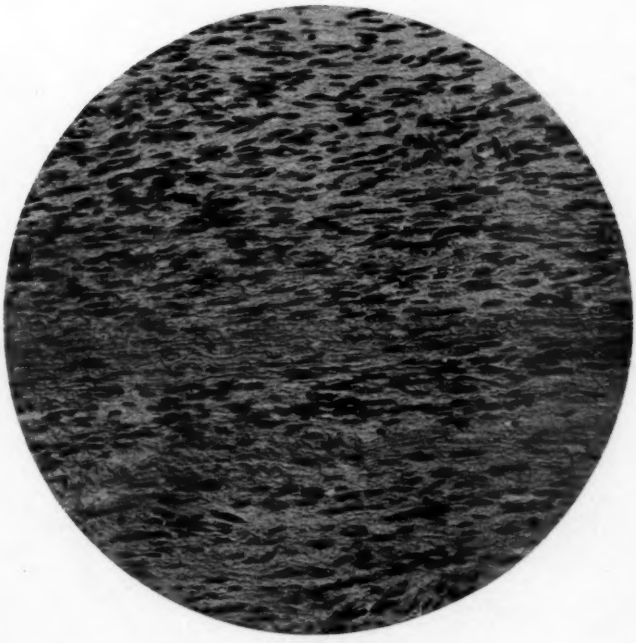

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Feldman

Leiomyosarcoma of Spleen in a Bovine

PLATE 34

FIG. 3. Leiomyosarcoma of the spleen. One cell in mitosis is shown. × 950.

FIG. 4. Metastatic leiomyosarcoma of the liver. Several cells in mitosis are shown. × 770.

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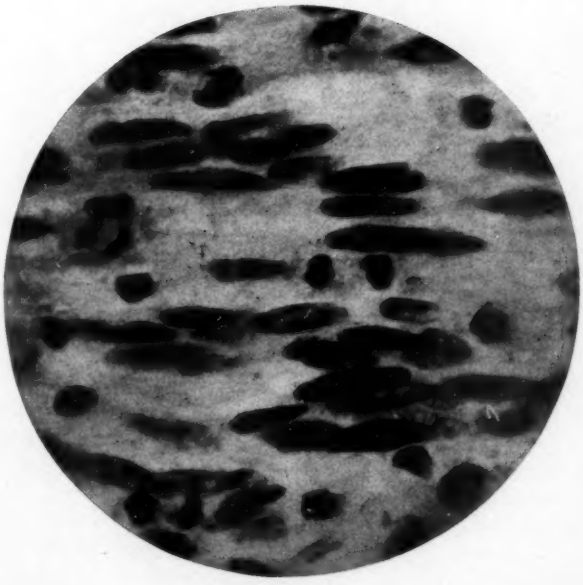

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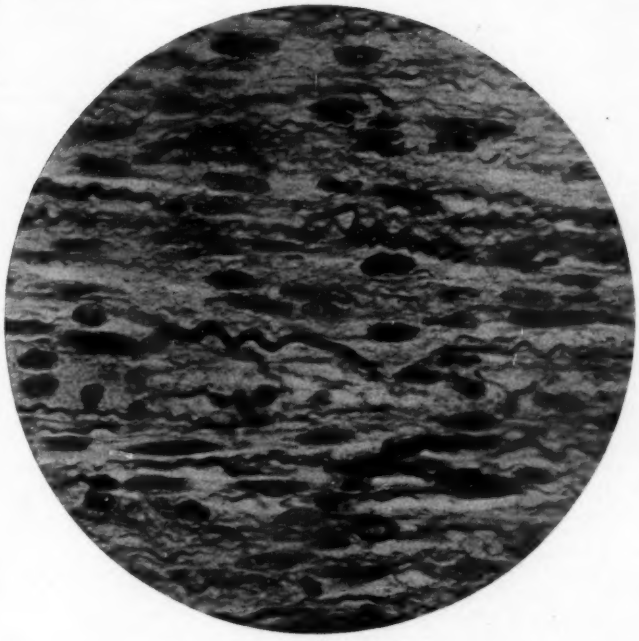
Feldman

Leiomyosarcoma of Spleen in a Bovine

PLATE 35

FIG. 5. Metastatic leiomyosarcoma of the liver. The wavy dark staining connective tissue fibrils are apparent. Van Gieson's stain. × 490.

FIG. 6. Metastatic leiomyosarcoma of the liver. The remaining hepatic cells are flattened and atrophic while the neoplastic elements show evidence of scirrhous change. × 175.



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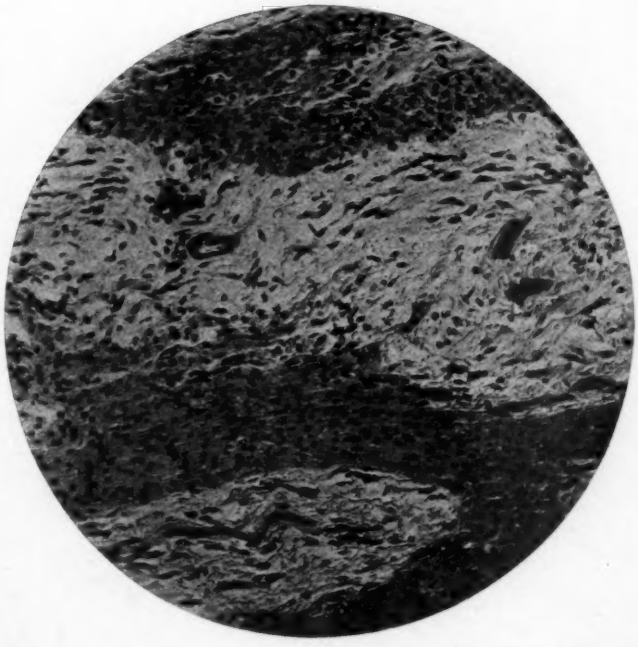

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Feldman

Leiomyosarcoma of Spleen in a Bovine

PLATE 36

FIG. 7. Metastatic leiomyosarcoma of the liver. Cross-section shows extensive involvement of the organ by metastatic nodular masses.

7

Feldman Leiomyosarcoma of Spleen in a Bovine

OBSERVATIONS ON THE HISTOLOGY OF THE TUMORS OF THE NERVUS ACUSTICUS*

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For many years the proper classification of the circumscribed, encapsulated, slowly growing tumors involving the sheaths of the central and peripheral nervous systems has been a subject for discussion. This group of tumors includes the so-called dural endothelioma or meningioma, the acoustic neuroma, the solitary fibroma of peripheral nerves, and multiple neuromas or Von Recklinghausen's disease. Some authors have felt that each of the foregoing represented a distinct entity. Others, notably Cushing,[1] have advanced the opinion that all should be included under one heading.

Thorough descriptions have been written of all the neoplasms. The solitary tumors of the eighth cerebral nerve, the so-called acoustic neuromas, have received in recent years rather less attention than the other newgrowths of this general class. Pathologists have not agreed as to their origin, and therefore, the type cell and the proper classification have been open to question. For these reasons it was thought worth while to subject a series of acoustic nerve tumors to careful microscopic observation with varied histologic technic to determine if possible in what general class they should be placed.

The tumors of the acoustic nerve have been classified in a variety of ways. They have been called gliomas, neuromas, endotheliomas and fibromas. The authors with conflicting opinions may be separated into two main classes: first, those who consider the growths to be derived from nervous tissue and second, those who hold that they take their origin from connective tissue.

The adherents of the first theory draw their deductions from three lines of reasoning, the first being simply that the microscopic appearance of the tumors is not typical of that seen in ordinary fibromas. They state that their peculiarities of structure, such as whorl formation and palisading of nuclei, are not seen in fibromas.

* Received for publication January 12, 1928.

From that fact they argue that the tumor is one of a different sort of cell, probably neuroglial in nature.

The French investigators, Lhermitte and Guccione,[2] represent the second point of view. They stained sections of these tumors with Victoria blue and demonstrated fibrils which colored sharply. As this dye stains neuroglial fibrils well they supposed that this evidence was proof of the origin of the tumors from neuroglial cells. It has been found, however, that Victoria blue stains fibroglia fibrils and fibrin as well as the fibrils of neuroglia. In a publication by Roussy, Lhermitte and Cornil [3] the view was also advanced that the tumors are gliomatous in nature.

The third argument is a very important one based on the embryologic work of Harrison, [4] and Harvey and Burr.[5] The first showed that when the neural crest is removed from frog embryos of a certain age peripheral nerves develop without a sheath of Schwann. Harvey and Burr demonstrated that the brain of an amblystoma embryo transplanted without the neural crest into a second embryo developed without the formation of a pia-arachnoid. If the neural crest was transplanted with the brain the leptomeninges developed normally. Thus there is strong evidence that the sheath of Schwann and the pia-arachnoid are derived from the neural crest and are ectodermal in origin. Verocay [6] suggested that the tumors of the acoustic nerve might arise from the sheath of Schwann and applied the term "Schwannoma" to them. If this view is accepted it follows from the work of the embryologists that the tumors should be regarded as of ectodermal origin.

This line of reasoning is open to attack on three grounds. First, Weed [7] has demonstrated that the pia-arachnoid forms in transplanted embryo brains only when choroid plexus as well as neural crest is included in the transplant. The failure of Harvey and Burr to grow meninges on a brain transplanted without neural crest may have been due as much to the absence of choroid plexus as of neural crest.

The second reason for doubt is the claim made by many authors, notably Mallory [8] that the tumors arise, not from the Schwann sheath but from the perineurium. This structure is considered fibroblastic in nature by most anatomists. Penfield [9] speaks of impregnating the fibers of the perineurium by a specific silver carbonate technic for staining collagen.

A third explanation is that the neural crest may contain undifferentiated mesenchymal cells which might give rise to the nerve coverings. There is a group of anatomists who maintain that connective tissue may develop from ectoderm as well as from mesoderm.

The opinion that these tumors are fibroblastic in nature was early voiced by Cushing, who stated that the type cell was probably a transition between neuroglia and connective tissue. Mallory took a very decided stand in favor of the fibroblastic origin of these neoplasms. He demonstrated the presence of fibroglia and collagen fibrils and felt that they should be classed with the tumors of peripheral nerves under the heading of perineural fibroblastoma. His opinion was that both of these growths arose from the fibrous perineurium.

Penfield employs the term perineural fibroblastoma as the result of careful studies using the various specific silver impregnation methods of the Spanish school of neuropathologists as well as routine stains. He states that he has never seen evidence of nervous or neuroglial elements in this type of tumor. This would seem to be a very definite reason for excluding these growths from the class of neuromas or gliomas. He does not state whether he has seen fibroglia or elastic fibrils in these growths although he does mention that he has frequently observed them in the dural endotheliomas.

In general, five criteria are used to prove that a given tissue is derived from the fibroblast. These are: (1) the morphology of the cell; (2) the presence of collagen; (3) of elastic fibrils; (4) of fibroglia fibrils, and (5) of reticulum. The last may need some explanation. Mallory and Parker[10] have recently shown that the fine fibrils impregnated with reduced silver in the various methods for the staining of reticulum are not fibrils of a specific nature. They are simply very fine strands of collagen which are separated enough by cells or by edema to allow penetration by the silver. Thus the formation of reticulum by a tumor means only that the cells form delicate, discrete fibers of collagen.

A tissue which can be shown to fulfill all of these requirements would be considered by most histopathologists to be fibroblastic in nature. We shall take up these points in order and show that all of the necessary constituents may be found in the tumors in question.

Technic: Fifty-four specimens were examined. All came from

the surgical clinics of Dr. Harvey Cushing. A few were sent to Dr. Mallory years ago from the Johns Hopkins Hospital and the remainder were obtained since then from the Peter Bent Brigham Hospital. Both Zenker's fluid and formalin were used as fixatives. The most important detail in this and other studies is to have absolutely fresh tissue cut into very thin pieces and placed immediately in a large amount of Zenker's fluid. The container should be shaken frequently to insure the contact of fresh fixative with the specimen.

The tissues were embedded in paraffin and cut at six microns. The sections were stained with eosin-methylene blue, phosphotungstic acid hematoxylin, Mallory's aniline blue collagen stain and neutral ethyl violet-orange G. Both the silver carbonate and silver oxide methods of Foot [11,12] were employed for staining reticulum. Bielschowsky's method for neurofibrils was used. The elastic fibrils were stained by the methods of Verhoeff and Weigert.

Morphology: As described by Cushing the tumors are composed of two types of tissue, one made up of dense interlacing fibrous bands and the other of loose reticular tissue. The first type is composed of a mass of elongated cells with oval nuclei of moderate size containing a rather small amount of evenly distributed chromatin. Palisading and whorl formation of the nuclei are often well marked. Between the cells and parallel with them run innumerable delicate wavy fibrils which take a light brown color with phosphotungstic acid hematoxylin and pale blue with Mallory's aniline blue collagen stain. Their color is pink when stained with eosin-methylene blue. As these fibrils become more closely packed together a darker and stronger brown to red color is developed, exactly like that given by collagen in general when stained by phosphotungstic acid hematoxylin. The reticular tissue is made up of elongated cells of a similar type but more or less separated from each other. The cell processes anastomose, forming a loose network. In the spaces so formed can be demonstrated wandering phagocytic cells, many of which contain fat. The loose tissue is very likely to contain precipitated albumen and fibrin, also at times blood or blood pigment. This tendency to degeneration is very likely to complicate the picture and make the determination of the true structure difficult.

There is a well marked stroma of fairly heavy connective tissue strands carrying with them fair sized vessels. In this stroma isolated fibroblasts are often found which sometimes contain a well

marked nucleolus. It is possible that these have at times been mistaken for nerve cells but we have nearly always been able to demonstrate fibroglia fibrils on them. True nerve cells which impregnate by the Bielschowsky method are extremely rare but are occasionally present. When nervous elements are present they probably have been included in the tumor in its growth since they are entirely different in morphology from the characteristic tumor cells.

Collagen: The background of the tumor is made up of parallel strands of fine wavy fibrils as previously described. When well separated they take a light brown color with phosphotungstic acid hematoxylin but when they are closely packed, the bright red-brown, characteristic color of collagen appears. With the other stains these fibrils take the color of fine strands of collagen.

Elastic fibrils: Only a few of the tumors show elastic fibrils in the tumor itself, although they are common enough in the stroma. When present they are unmistakable. The arrangement is a branching, anastomosing network of fine to thick, irregular strands running at various angles to the predominating direction of the tumor cells. There are a number of fibers of similar shape and arrangement present in these tumors which stain lightly or not at all by the various methods for specifically coloring elastic fibers. They stain sharply by phosphotungstic acid hematoxylin and neutral ethyl violet-orange G. These we take to be poorly developed fibers of the same type.

Fibroglia fibrils: These are the most difficult to find of any of the intercellular products of these tumors. The most exhaustive search was required to find fibers coarse enough to be suitable for photography. Fibrils, staining properly, and of the required morphology and arrangement, were present in abundance in many of the tumors but were too delicate to photograph distinctly.

Reticulum: By proper methods of silver impregnation abundant fibrils may be shown, thickly packed and running parallel to the cell bodies. By careful comparison with preparations stained by other methods they can be shown to correspond exactly to the pale staining wavy fibrils which form the mass of intercellular substance of the tumor and which we have described under the heading of collagen.

Discussion

From the foregoing findings the pathologist would have no hesitation in saying that these tumors are of fibroblastic nature. The embryologic evidence is very hard to explain unless we assume that it does not apply to the development of the perineurium and that the tumors do develop from this structure. Without entering that phase of the subject we can say only that by all the ordinary criteria the tumor is fibroblastic in nature. If the neural crest gives rise to the perineurium as well as the sheath of Schwann it may be possible that undifferentiated mesenchymal cells are present in it and from them the perineurium develops. At all events, whatever the embryology may be, the morphology of the cells and the chemical reactions of the intercellular products are those of connective tissue. In view of these facts we feel that they should be classed with the perineural fibroblastomas and not with the gliomas.

Summary

1. The type cell of the solitary tumor of the acoustic nerve, the so-called acoustic neuroma, forms collagen, elastic and fibroglia fibrils, also reticulum.

2. The histologic characteristics of the tumor are consistent with those of newgrowths derived from connective tissue.

3. The development of the characteristic fibrils is not so well marked as in connective tissue growing in other tumors.

Conclusion

The solitary tumor of the eighth cerebral nerve, the so-called acoustic neuroma, should be classed as a perineural fibriblastoma.

We are indebted to Miss Lillian M. Leavitt for histologic aid and to Miss Catherine G. Norton and Dr. F. B. Mallory for the photomicrographs.

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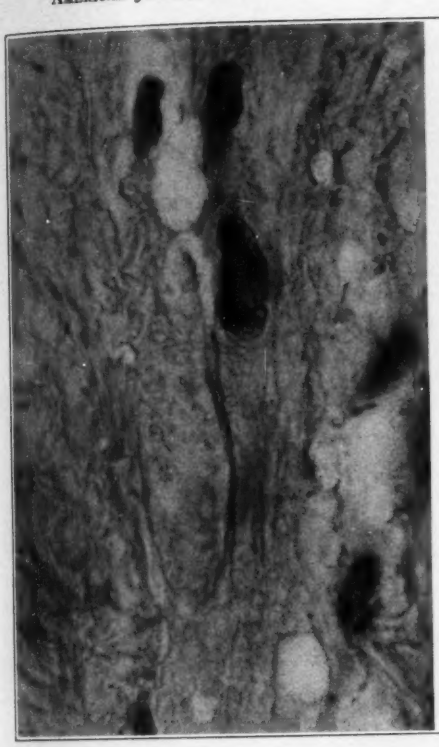
DESCRIPTION OF PLATES

PLATE 37

FIG. 1. Photomicrograph showing delicate fibroglia fibrils on either side of a characteristic cell of an eighth nerve tumor. × 1500.

FIG. 2. Section cut across a bundle of tumor cells showing fibroglia fibrils in cross-section. × 1500.

FIG. 3. Fibroglia fibrils in an arachnoid fibroblastoma for comparison with those in a perineural fibroblastoma. × 1000.



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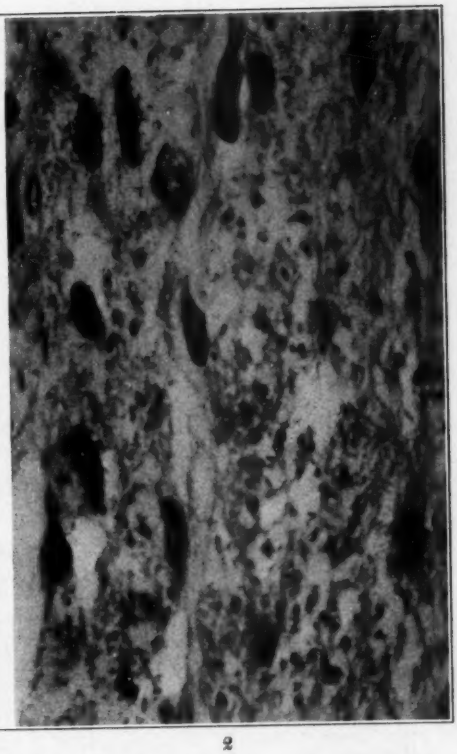

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Plate 38

Fig. 4. Section of a perineural fibroblastoma showing several tumor cells with fibroglia fibrils. × 1500.

Fig. 5. Showing a fibroglia fibril passing over the nucleus of a tumor cell. Other fibrils show less distinctly between the other cells. × 1500.

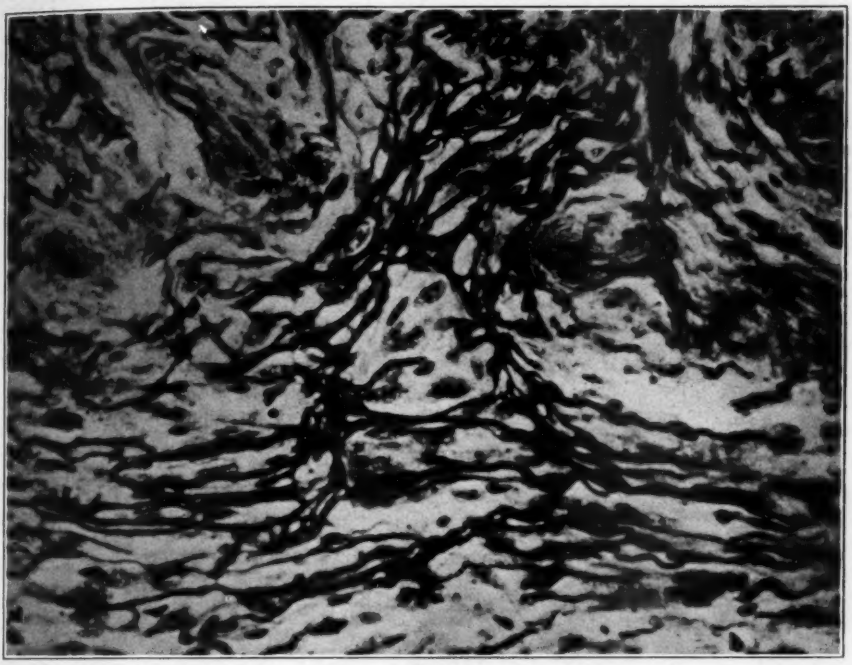
4

5

PLATE 39

FIG. 6. Dense network of coarse elastic fibers in a characteristic area of a perineural fibroblastoma. × 500.

FIG. 7. Reticulum in an area of the tumor where the structure is loose and infiltrated with endothelial leucocytes. × 500.



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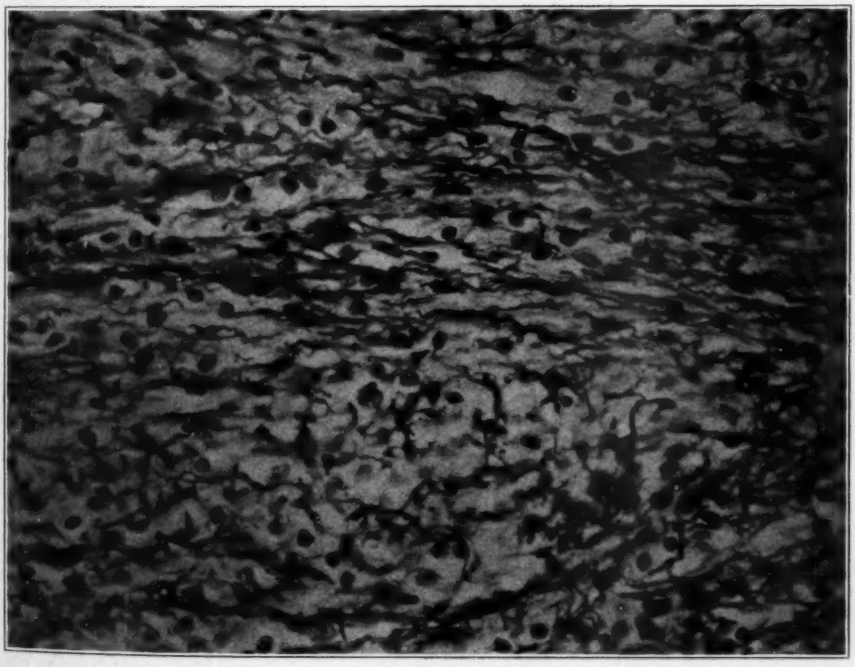

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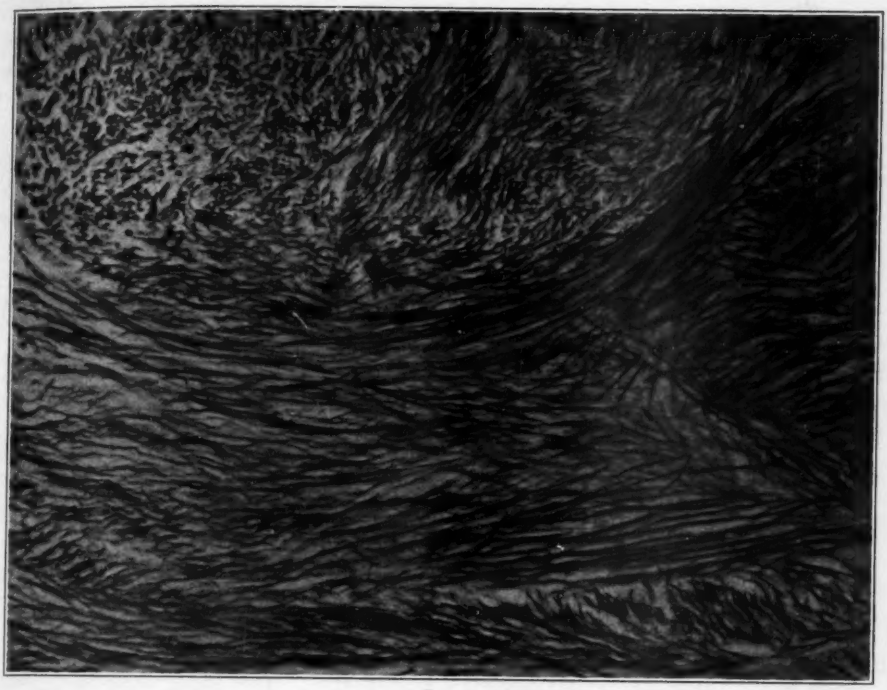
PLATE 40

FIG. 8. Reticulum in an area of the tumor where the structure is more dense. The fine fibrils which make up the background of the tumor impregnate sharply. × 500.

FIG. 9. Fibroglia fibrils on two fibroblasts in the stroma. The nucleoli are well marked. × 1500.



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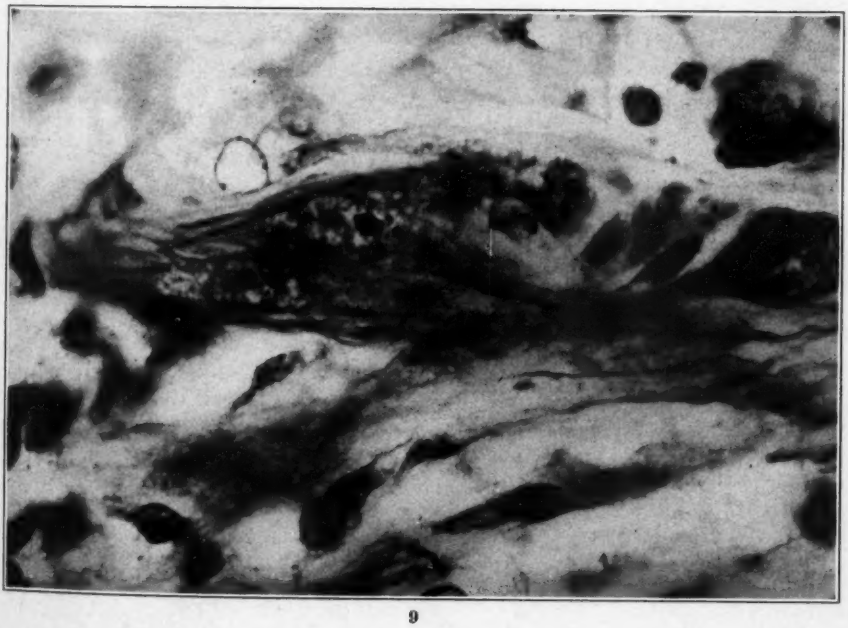

9

A METHOD OF STAINING OLIGODENDROGLIA AND MICROGLIA (COMBINED METHOD) *

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The smaller cells of the central nervous system were able to hold back the secret of their form for many years in spite of the variety of methods used to stain the other cellular elements. Robertson [1] revealed the cytoplasmic expansions of oligodendroglia with platinum but the method was so unreliable that it never found its way into laboratories outside of Edinburgh.

Most methods stain the nuclei of microglia and oligodendroglia and by Nissl's method the cytoplasmic bodies are often indicated, but not the expansions. Del Rio-Hortega [2] stained and described microglia completely by silver carbonate and later, by a small modification of the same method, he also revealed the structure of oligodendroglia.[3]

Silver carbonate may be used to demonstrate microglia (Hortega's cells) even after they have been changed into the rod cell form or transformed still further into compound granular corpuscles.[4] In this phagocytic state these cells, after being stained with silver, may be readily counterstained by the commoner methods for fat.

When oligodendroglia has undergone acute swelling either during life or as a postmortem change a successful preparation shows the cell body to be swollen with unstained fluid, the expansions to be fragmented and these fragments often similarly distended.[5] Autopsies must be early to avoid such change in these cells as an autolytic process. But it also occurs during life in various types of intoxication.

The method of Del Rio-Hortega necessitates primary fixation in formalin-ammonium-bromide solution. This is true also of the alcohol modification of this method.[3] In a paper before the American Neurological Association in 1925, Globus [6] provided the key to

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the utilization of formalin material for the gold and silver stains which require ammonium bromide as mordant. At that time he proposed a modification of Cajal's gold chloride sublimate method for neuroglia. He removed the excess formalin from the sections with ammonia after the custom of Del Rio-Hortega, but left them in ammonia a longer period and then placed the sections in hydrobromic acid before proceeding with the impregnation. This admirable variation will sometimes yield excellent results even in old material.

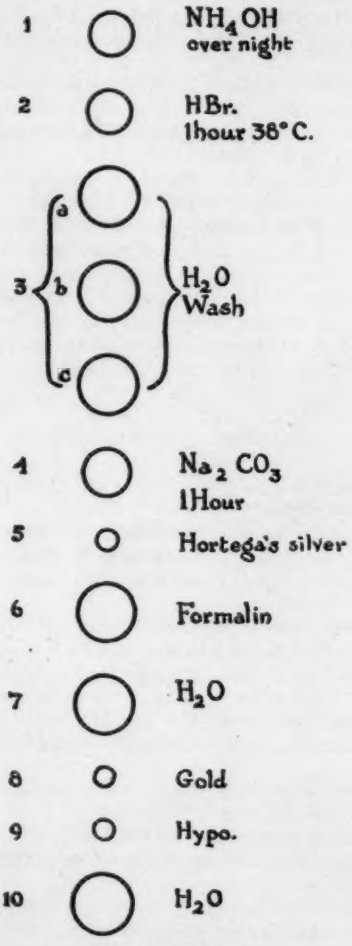
In our laboratory Dr. M. Fulstow began at once to apply this maneuver to Del Rio-Hortega's method of staining microglia and oligodendroglia. She found that successful results could also be obtained for these cells if sodium carbonate be used between Globus' hydrobromic acid and Hortega's silver. In a long series of trial variations during the past year we have standardized the technic as far as we were able.

Consequently, in numerous minor, though important details the method, as it is used at the Presbyterian Hospital, differs from that published by Dr. Globus. A descriptive note, therefore, seems justifiable particularly as the method has proved to be a combined one demonstrating both microglia and oligodendroglia neither of which could be stained in our hands with the procedure outlined by Globus.[6] Moreover to judge by the number of inquiries which have reached us concerning the technic of demonstrating these cells, there is a rapidly growing interest in them.

The results by this method are not better than those obtained by the original Hortega methods when the latter are successful. But the new procedure is more consistently successful with pathologic material and primary fixation in formalin provides tissue for other stains. Very good results are obtained if blocks be taken from the brain after hardening for five days in 10 per cent formalin. The staining is better and the cytologic preservation improved if the brain is originally injected with formalin. Merck's neutral blue label formalin gives better results than some other formalins in our hands. Doubly distilled water should be used for all solutions and the glassware must be clean.

If successful, both types of cells are well stained throughout but inequality in the perfection of impregnation throughout a single section may be seen. For example, microglia may stain best in

the gray matter and oligodendroglia in the white matter. Because of such variations in a single preparation, sections may be mounted after varying lengths of time in silver. It is of cardinal importance



TEXT-FIGURE 1

that the worker carrying out the technic shall be able to recognize the cells in question on the wet slide before mounting and that he be familiar with the typical pathologic alterations of these cells.

The Method

1. *Harden:* Tissue in 10 per cent formalin (or formalin-ammonium-bromide) for an indefinite period. About a week in formalin gives excellent results.
2. *Section:* Cut sections at 20 microns on the freezing microtome and receive them in 1 per cent formalin or distilled water. Through the succeeding steps the sections should be handled by a glass rod shaped like a hockey stick.
3. *Deformalinize:* Place sections in dish of distilled water to which 10–15 drops of strong ammonia have been added and cover so as to prevent escape of ammonia. Leave in this solution over night to remove formalin (see Text-Fig. 1, No. 1).
4. *Bromurate:* Transfer sections directly to Globus' hydrobromic acid in 5 per cent solution (5 cc. of 40 per cent hydrobromic acid plus 95 cc. distilled water). Place in incubator at 38° C. for one hour.
5. *Wash:* Pass through three changes of water (a, b, c).
6. *Mordant:* Place sections in 5 per cent solution of sodium carbonate for 1 hour. (Sections may remain here 5 to 6 hours without ill effect.)
7. *Impregnate:* Pass sections with or without washing direct to Del Rio-Hortega's silver carbonate, weak solution,* and leave them here 3–5 minutes. Sometimes they may be left till they begin to turn a yellowish gray. Then transfer them to the reducer. Control the duration in silver solution by taking out a section at intervals of 1–2 minutes and examining under the microscope. The sections should turn a smooth gray color in the following reducer.
8. *Reduce:* Plunge into 1 per cent formalin and agitate.
9. *Wash:* Distilled water.
10. *Tone:* Leave in gold chloride † (1–500) at room temperature until all yellow tint disappears and the sections are a smooth bluish gray.
11. *Fix:* Hyposulphite of soda (5 per cent photographic "hypo").
12. *Wash:* Distilled water.
13. *Dehydration* may be done conveniently after Del Rio-Hortega's custom as follows: Float sections on to slide and flatten out with needle. Wash with two to four changes of 95 per cent alcohol from a drop bottle. Follow this with a few drops of carbol-xylol-creosote (proportion of 1–15). When clear, drain slide and blot immediately with two thicknesses of fine filter paper. Mount in Canada Balsam.

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* Del Rio-Hortega's ammoniacal silver carbonate (weak solution)

Silver nitrate (Merck), 10 per cent solution	5 cc.
Sodium carbonate (pure), 5 per cent solution	20 cc.
Ammonium hydroxide (sufficient to dissolve the precipitate)	
Distilled water, ad	75 cc.

The ammonium hydroxide in strong, fresh solution should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. It is important not to add too much ammonia. A fine black sediment may remain behind which does not resemble the more voluminous precipitate of silver carbonate. This fine sediment should be filtered off. The solution may then be preserved in a dark bottle for long periods.

† The yellow variety of gold chloride is less expensive than brown gold and seems to be preferable for toning.

By this method microglia and oligodendroglia may be stained with a considerable degree of consistency. The morphologic differences of the two types of cells make it quite easy to distinguish them. This differentiation is made even simpler when either type of cell has undergone some pathologic change. For example: a generalized toxic condition, or autolytic and postmortem influence may cause acute swelling of oligodendroglia [5] while microglia is left unaltered.

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AN INFLAMMATORY BASIS FOR CORONARY THROMBOSIS *

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Occlusion of a coronary artery is almost always due to a thrombus or an embolus. Of the twenty-three cases of occlusion reported by Wolff and White [1] nineteen were due to thrombosis and four to embolism. The usual sources of emboli were either a mural thrombus of the left ventricle or a vegetative endocarditis. Thrombosis never occurs in a healthy vessel, while emboli may occlude normal coronary arteries. In Hamman's [2] review of the relative frequency of thrombosis and embolism, he reports that Longcope had sixteen cases of thrombosis and one occlusion by embolus; that Faulkner, Marble and White had twenty-five cases of thrombosis showing arteriosclerosis and one vegetative endocarditis with embolism; that LeCount had twenty-nine cases of thrombosis and one of endocarditis with embolism.

Wolff and White state that "the most commonly involved vessel is the anterior descending branch of the left coronary artery, but any of the other branches may be the seat of the occlusion." Of their nineteen cases of thrombosis the left coronary was involved in thirteen, the right in four, both in one case; and the remaining one was an unusual case with the occlusion at the root of the aorta. Wearn [3] reports that sixteen out of his nineteen cases showed the occlusion in the anterior descending branch of the left coronary, one in the posterior descending branch, and one in the posterior circumflex branch of the left coronary. In the review by Hamman, referred to above, he reports that of thirty cases the anterior descending branch of the left coronary artery was occluded in twenty-two cases, the orifice in two and the left circumflex in one case, while the right coronary artery was involved in five cases. Of course not all of these cases showed thrombosis. Twenty-five showed arteriosclerosis, four syphilitic aortitis, and one an embolus. We see from these records that occlusion occurs most frequently in

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the anterior descending branch of the left coronary artery and that the occlusion is almost always due to thrombosis.

In practically every case of coronary thrombosis described there has been generalized arteriosclerosis or at least sclerosis of the coronary arteries. However, in two of Herrick's [4] cases the only significant vascular sclerosis was in the coronary arteries. Riesman [5] states that the etiology of coronary thrombosis coincides with that of coronary sclerosis. According to Wearn, in addition to the coronary arteries which were markedly sclerosed in every case, there was sclerosis of the aorta varying from mere fatty plaques to extensive atheromatous ulcerations with calcification. Hamman found that the chief cause of occlusion was arteriosclerosis and the final closure was usually due to thrombosis. He concluded that arteriosclerosis alone would cause such gradual obstruction that a compensating collateral circulation would develop so as to prevent alarming symptoms. But when the vessel has become markedly diseased, even before the inner walls have approximated, thrombosis usually occurs to occlude the vessel abruptly. Gordinier [6] also mentions the atheromatous changes in the coronary arteries associated with coronary occlusion. Wolff and White state that "the coronary arteries in all cases of thrombosis are sclerosed, and usually narrowed." They agree with Hamman as regards the arteriosclerosis and narrowing of the lumen, and add that "an exceptional case may show only very slight fibrotic changes, but apparently the injured intima in such instances is sufficient to allow thrombosis to occur." Willius [7] says that patients dying of coronary thrombosis always show arteriosclerosis of the coronaries. He adds that the aorta is also the seat of disease, consisting of sclerosis, atheroma and ulceration.

The thrombus usually forms at a point where the sclerotic changes have caused considerable narrowing of the lumen. This point is usually at a variable distance from the orifice but is not far from the bifurcation of the descending branch of the left coronary. In fifteen out of Wearn's sixteen cases the thrombus was situated on the site of a contracture in the lumen due to an atheromatous change in the vessel wall. The thrombus was not found in one of his cases but the lumen of the artery was practically closed at one point by an atheromatous thickening. One of Paullin's [8] cases showed thrombosis in the anterior descending branch of the left coronary and

another in the posterior circumflex of the left coronary. The thrombi formed over the calcareous plaques caused infarcts near the tip of the left ventricle and the posterior part of the left ventricle, respectively. The occlusion by a coronary thrombus usually occurs at only one point but more than one vessel may be affected.

It appears that in most cases of coronary thrombosis there is an underlying arteriosclerosis forming the basic lesion upon which the thrombus is formed. Little attention has been paid to the immediate changes which precipitate the deposition of thrombus. It is evident that a coronary artery may remain sclerosed for years without thrombosis and there must be some mechanism which eventually incites the deposit of platelets and fibrin.

This phase of the question has been considered, especially in the two cases to be described, in which the mechanism is clearly in evidence. An arteriosclerotic plaque may lead to sudden thrombosis whether it be situated in the aorta or in a coronary artery by reason of the onset of an acute inflammatory change within the plaque. The cause of the acute inflammation which may occur in atheromata is obscure, but the following cases suggest vascular injury about the atheroma, possibly from circulating toxic material derived from an extensive infection, as one agency in its production.

The outpouring of exudate into an atheromatous plaque distends it with serum, fibrin, red blood cells and white corpuscles. In the two cases studied here such an exudate and hemorrhage were very abundant, and they appear to have been superimposed upon some change, vascular or otherwise, which took place within the atheromatous areas not only in the coronary arteries but also in the aorta. Secondary to the inflammation and the distension of the sclerotic patches the fresh thrombus was deposited, effecting in the case of the coronary vessels immediate and complete occlusion.

CASE 1. The first case is a white housewife, aged 65 years who entered the hospital February 27, 1927 with signs of consolidation and fibrinous pleurisy of the lower right pulmonary lobe. The onset of this condition dated back two weeks before admission when the patient had a cold and cough, followed by shortness of breath, a tight feeling in her chest and some pain.

The course was relatively uneventful until March 3 when she complained of a severe pain in the chest extending across the upper anterior portion and into the right axilla.

Four days after admission the physical signs of a non-productive cough, a prolonged expiration with a grunt in association with rales, dullness in the right chest, suppressed breath sounds and a dry friction rub in the right axilla,

were not definitely changed; but the patient appeared more acutely ill. She vomited once. The leukocyte count rose from 9,000 to 11,000. The temperature which had ranged from 99° F. to 103° F. fell to normal during the evening of March 3 and was 98.2° F. at midnight, where it remained.

The following morning at 8.30 the patient appeared to be in pain but was breathing quietly. While she was under observation, her facial expression showed anxiety and suddenly became fixed, drawn and still. There was no gasping and no struggle. Respiration ceased and her pulse and heart beat were imperceptible.

The anatomic diagnoses are: thrombosis, coronary, left descending; atheromatous degeneration of coronary arteries; acute inflammation, atheromatous areas; necrosis, myocardial, acute; bronchopneumonia, acute, suppurative; hydrothorax, right; acute passive congestion, liver; passive congestion, kidneys, spleen, adrenals and mesentery.

Pathologic Findings: Sections through the descending branch of the left coronary show a large atheromatous plaque which has pushed the thickened intima far out into the lumen of the vessel. Within this atheromatous area there are numerous cholesterin crystal clefts which take various shapes, and some of them are actually pulled apart. There is an abundant content of serum within the plaque which has caused a swelling of the entire area with a pushing out of the intima, increasing the stenosis of the lumen. There are occasional leukocytes scattered through the atheroma and also some about the margin of the plaque. About the intimal and the lateral margins of this atheromatous plaque there are numerous small blood vessels, apparently branches of the vasa vasorum, grown about its margins. Large amounts of fibrin and numerous red blood cells are deposited all about these small vessels. This hemorrhage serves further to elevate the intima and thereby obliterate the lumen still more. The endothelial surface is irregularly roughened from injury, and a fresh thrombus is deposited upon it at the site of greatest obstruction. This completes the obstruction of the already narrowed lumen.

CASE 2. The second case is a white woman, aged 69 years, who entered the hospital with a fairly typical history of gall-bladder disease. She came back later with no improvement of her previous condition. Her blood pressure was systolic 170 and diastolic 72. The leukocyte count was 16,000. She was very jaundiced and this increased along with a rise in temperature. The abdomen became very rigid and about the same time the patient passed a large stone 3 cm. in diameter by rectum. She was operated upon the following morning.

The gall-bladder had perforated into the duodenum. Following the operation for removal of the gall-bladder she apparently reacted well. The temperature rose to 102° F. The wound drained a brownish fluid. At four o'clock in the morning on the ninth day after the operation the patient died. The type of death was not known but it was thought to have been very sudden and peaceful.

The anatomic diagnoses are: thrombosis, coronary, left descending; atheromatous degeneration of coronary arteries and aorta; acute inflammation, atheromatous areas; cholecystoduodenal fistula; peritonitis, acute suppurative, encapsulated; splenitis, toxic; passive congestion, liver, lungs, mesentery and intestines.

Pathologic Findings: The intima of the descending branch of the left coronary artery is irregularly thickened and a large atheromatous plaque pushes the superficial layers of the intima out into the lumen. There are numerous cholesterin crystal clefts within the plaque. There is a large amount of serum present which has caused great swelling and an increase in the size of the plaque with a pushing out of the intima, further obliterating the lumen. Within this edematous plaque about the cholesterin crystals there have accumulated numerous cells, many of which are polymorphonuclear leukocytes. This area is softened due to partial solution of the plaque. About the outer margins of the atheroma there are some small blood vessels with hemorrhage and fibrin deposited all about them. The endothelial surface is irregularly roughened due to some injury and a fresh thrombus is attached to the vessel wall. This thrombus completely obstructs the lumen which has already been narrowed.

Within the intima of the aorta there are large atheromatous plaques which cause the intima to stand out in an irregular manner. There are many cholesterin crystal clefts about which are many polymorphonuclear leukocytes and large mononuclear leukocytes, and a great deal of serum which has caused a swelling of the entire area. This raises the superficial layers of the intima. About the outer margins of the atheromatous plaques there are some small vasa vasorum around which are hemorrhage and fibrin. This hemorrhage and the deposition of fibrin serve to increase the volume of the atheromatous area and to extend the superficial layers of the intima still farther out into the lumen. The endothelial surface is irregularly roughened and a fresh thrombus is attached in places to the vessel wall.

Discussion

The acute changes which have taken place in the above described arteries leading to thrombosis are all superimposed upon a pre-existing arteriosclerosis. They have been found in the aorta and in the coronary vessels. The recent changes have been studied as they have occurred in two cases of coronary thrombosis, in one of which they were present also in the aorta.

The large atheromatous plaques form within the intima producing an irregular thickening which pushes out the superficial layers of the intima into the lumen of the vessel and thereby reduces its size. Superimposed upon these atheromatous areas are acute inflammatory changes. The plaques show the typical clefts that have been left by the cholesterin crystals which have been dissolved. About these irregular clefts serum is present in large quantities and this acute exudation has brought about a marked increase in the volume of the plaques. The increase of the volume in the atheromatous areas serves to extend the superficial layer of the intima still farther out into the lumen. In addition to the serous effusion there has been an exudation of polymorphonuclear leukocytes and large mononuclear leukocytes about the crystal clefts and an acceleration of the process of necrosis and softening. In association with the older sclerotic process numerous small vasa vasorum have grown in about the margins of the atheromatous plaques to supply the damaged wall of the vessel. There are large depositions of fibrin and extensive hemorrhages about these small vessels which indicate some injury to their walls. These areas of hemorrhage and fibrinous exudate begin about the small blood vessels and extend in toward the centers of the plaques and even out under the elevated intima. The volume of the plaques is thereby increased and the intima is raised farther and pushed out into the lumen. The acute inflammation and hemorrhage about the atheromatous areas are probably of very short duration.

The atheromatous plaque with its acute exudate and resulting softening, together with the hemorrhages about the outer margins, causes a great outpushing of the superficial layers of the intima into the lumen. The endothelial surface of the vessel becomes injured due to the various changes going on underneath it or to changes due to the circulating blood passing over the distended and irregular

intimal surface as it stands out in the lumen of the vessel. Upon this injured endothelial surface a fresh thrombus forms, which in the case of the coronary arteries further obliterates the lumen of the vessel or causes complete obstruction.

In both the cases described above there was an acute infection; in the one, an acute bronchopneumonia, in the other a suppurative localized peritonitis. The association of such infections with the acute degenerative and inflammatory process going on in the sclerotic vessels suggests a causal relationship. Infection has long been thought of as a possible etiologic factor in arteriosclerosis for one so frequently finds acutely developed fatty patches in the intima associated with various infectious diseases. That infections of various kinds are operative in acutely advancing and extending arteriosclerotic lesions, already existent but probably quiescent, is equally probable.

These two cases clearly demonstrate the fact, to which we have not found reference in the literature, that an arteriosclerotic plaque may suddenly undergo an acute degeneration associated with inflammation and that thrombosis may be the result. Such an exacerbation of an old arteriosclerosis may be general, that is, it may involve all the arteries affected, and in the case of the coronaries may lead to sudden death from an occluding thrombus.

Summary and Conclusions

1. Two cases of thrombosis of the left coronary artery are reported.

2. An acute inflammation of the atheromatous plaques appears to have been the immediate cause of the deposition of thrombus.

3. An extensive suppurative process in a person with arteriosclerosis may cause an acute exacerbation of the vascular lesions wherever they are situated, and in the case of the coronaries may lead to a fatal thrombosis.

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DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Coronary artery of Case 1. The atheromatous plaque is softened and partially liquefied. The dark areas are composed of fibrin and red blood corpuscles. There is a fresh thrombus attached to the wall of the vessel. × 20.

FIG. 2. Coronary artery of Case 2. The atheromatous plaque is softened and degenerated. The small black dots scattered throughout the plaque are polymorphonuclear leukocytes. The single irregular dark area near the margin is composed of fibrin and red blood cells. There is a fresh thrombus attached to the intimal wall of the vessel. × 20.

1

2

PLATE 42

FIG. 3. Section of aorta of Case 2. The large atheromatous plaque is softened. The dark areas are composed of fibrin, red blood cells and polymorphonuclear leukocytes. × 20.

FIG. 4. Another section of aorta of Case 2. The atheromatous plaque is softened and almost dissolved. The numerous small dots are polymorphonuclear leukocytes scattered throughout. The darker irregular areas are fibrin and red blood cells. × 20.

3

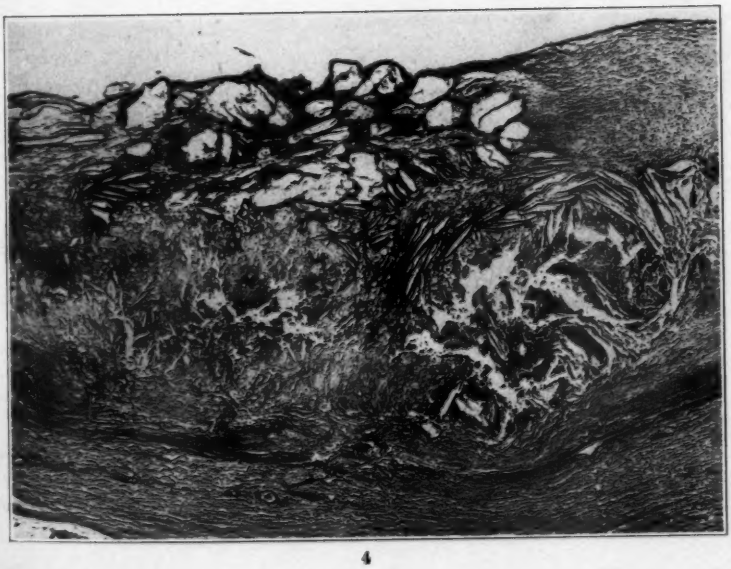

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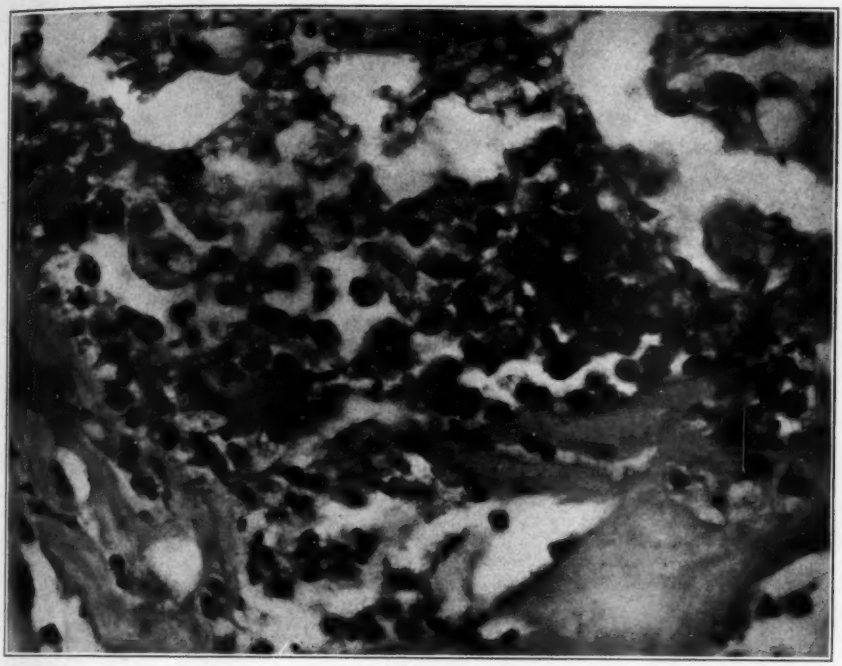
PLATE 43

FIG. 5. A high power view of an atheromatous plaque in a coronary artery of Case 2. The dark cells are all polymorphonuclear leukocytes. × 800.

FIG. 6. A high power view of an atheromatous plaque of the aorta of Case 2. Shows the numerous polymorphonuclear leukocytes scattered throughout in an area of softening. × 800.



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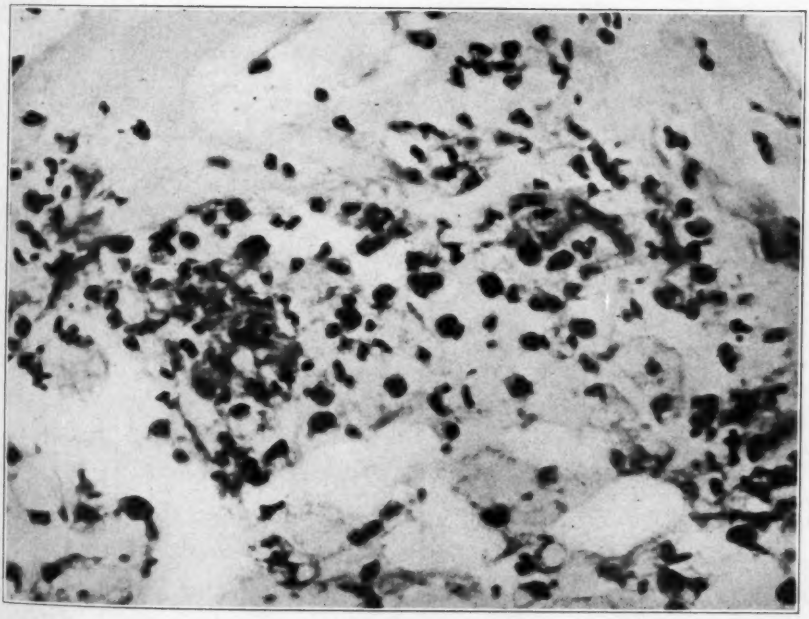

6

SOME OBSERVATIONS ON INCUBATED LEUKEMIC BLOODS *

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The observations reported in this paper were made during the course of a series of studies of human and animal bloods, exudates and tissues. The purpose of these studies was to gain additional information concerning the origin and physiological activities of the mononuclear phagocyte about which so much has been written in recent times. Leukemic blood was chosen for one set of experiments because of its content of certain types of cells in large numbers. Cultures of normal blood have been reported in detail by M. Lewis,[1] W. Lewis,[2] Carrel and Ebeling,[3] while Awrorow and Timofejewskij [4] described cultures of blood from cases of myelogenous leukemia. Our results agree very closely with those of the last mentioned authors. Since we were able to employ a more modern and varied technic for the study of the cells we felt that it was worth while to describe our observations. We have not used the word culture in describing our work for we did not have sufficient evidence that the cells under the conditions of our experiments did more than merely survive without undergoing multiplication.

TECHNIC

The blood was obtained in every instance from the median basilic vein. Supravital preparations and fixed smears were generally made before incubation.

The majority of workers in culturing blood have used a solid medium, usually clotted plasma. The drawback to such a medium is that in any one specimen the cells can be observed only once after staining. We felt that if a fluid medium could be employed from which samples could be repeatedly removed the results obtained would be more satisfactory and uniform; the cells would come from the same specimen and would be under the same conditions from day to day. A brief description of the methods employed is given:

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1. *Clotted blood.* One or two c.c. of whole blood was put in test tubes, allowed to clot, then rimmed and incubated. This method was used by M. Lewis at times as a control on her coverslip clot cultures.

2. *Citrated blood.* Blood was diluted with an equal volume of one per cent sodium citrate in Locke's solution. If the blood was incubated in this condition the cells died in a short time (one to two days); it was found that if the citrated blood was centrifuged and one or two drops of the sediment added to one or two c.c. of human or sheep serum the cells survived much longer.

3. *Heparinized blood.* Blood was added to a 1:200 solution of heparin in Locke's solution so that the final concentration of heparin was 1:600 or 1:800. As in the citrated blood the cells died rapidly if the blood was incubated in this state. Therefore, with or without centrifuging, one or two drops of heparinized blood were added to one or two c.c. of heparinized human plasma. The cells under these conditions usually did well; they did even better if several (seven to ten) volumes of Locke's solution were added to a combination consisting of equal volumes of heparinized blood and heparinized plasma.

The test tubes used measured 100 × 13 mm. The tops of the cotton plugs were cut off and the tubes were sealed with paraffin and incubated at 37.5° C.

Specimens for examination were removed with a platinum loop. These specimens were studied in three ways: — the supravital method as described by Sabin, Doan and Cunningham;[5] by Wright's blood stain; and by Sato and Sekiya's[6] peroxidase stain on dried smears.

Material

Eight cases of myelogenous leukemia and seven cases of lymphatic leukemia were utilized in this study. The total leucocyte counts varied from 6,000 to 226,000 in the myelogenous leukemia cases, and from 2,500 to 400,000 in the lymphatic leukemia cases. The patients were at different stages of the diseases and had had various treatment. At this point, we should like to express our gratitude for material to Dr. George R. Minot and Dr. Henry Jackson, Jr. of the Collis P. Huntington Memorial Hospital, Boston, and to Dr. Ralph C. Larrabee of the Boston City Hospital.

Observations

The results of the different experiments in each group were sufficiently similar to allow a composite description. Each case could be discussed separately but this would entail needless repetition and would be of no advantage. Any significant deviation from the average will be noted. The results of the myelogenous leukemia cases will be taken up first.

Myelogenous Leukemia

The methods of treating the blood have been described above. In each instance, tubes of clotted blood were used and in addition one of the other methods. Of the eight cases, five gave best results in the clotted blood tubes, while the remaining three were most satisfactory in the heparinized blood, heparinized plasma tubes. The periods of survival of the cells were the criteria for judging the results. The character of the cells when in good condition did not differ materially in the various media. The description in each experiment is based on the most satisfactory medium for that particular case.

The word "monocyte" is used in the following descriptions for the mononuclear as described by Sabin, Doan and Cunningham. This cell, as described by these authors, in vital preparations shows the following characteristics: — it is a cell with a single round or indented nucleus; the cytoplasm contains near the nucleus or in its hof a collection of rather fine, salmon-pink granules, often arranged around a clear central area; such an arrangement is known as a rosette; mitochondria occur at the periphery of this rosette and around the nucleus. Phagocyted material is always placed at the periphery of the cell, never in the hof of the nucleus as in the clasmatocyte. It is a fairly actively motile cell, less so than the polymorphonuclear neutrophile and more so than the lymphocyte. This cell corresponds to the monocyte of Naegeli [7] — a cell larger than a polymorphonuclear leucocyte with a lobed or indented nucleus and a steel or dusty gray cytoplasm containing numerous granules; the great majority if not all such cells in human blood are oxidase positive.

Polymorphonuclear neutrophiles. While many cells of this type are dead after twenty-four hours of incubation, there are still

many perfectly normal in appearance and motility; some contain a few refractile granules and occasionally a globule staining with neutral red; with the Wright's stain such refractile granules or globules appear as round, clear vacuoles. The dead cells as well as the living give a positive result with the peroxidase reaction. After forty-eight hours, while many still appear normal, an increased number over the previous day show refractile granules and vacuoles. A few contain phagocyted material in addition. At seventy-two hours, still more show the changes described above although there are a considerable number of unchanged cells. From that time on the number of living cells decreases as does the number of those of normal appearance. As a rule, no living cells of this type can be found after two weeks incubation. They always give a positive peroxidase reaction; it is interesting to note that they continue to do so even after they are dead and markedly degenerated.

Polymorphonuclear eosinophiles. This type of cell survives constantly longer than the neutrophile. Living cells are found as late as the twenty-first day. They retain their normal appearance throughout life although occasionally they show a small clear vacuole in fixed smears. They undergo none of the degenerative changes seen in neutrophiles but apparently remain typical until dead. Phagocytosis is never seen. The cell does not increase or diminish in size. The peroxidase reaction is always strongly positive.

Myelocytes. Living cells of this type can be recognized for about as long a period as the polymorphonuclear neutrophiles. Whether they survive longer but in so atypical a form that they cannot be recognized is possible, as will be seen under discussion of the "X" and "Y" cells. While they can be followed, an increasing number show in the vital preparations refractile granules at the periphery of the cell; these granules are usually smaller than those seen in the polymorphonuclear neutrophiles. Stained by Wright's, the cytoplasm contains round, clear vacuoles, and in some is entirely vacuolated. The peroxidase reaction remains positive even after death. In a few instances, typical myelocytes are seen containing phagocyted red blood corpuscles. Whether this type of cell enlarges or changes into an unrecognizable form cannot be stated definitely at the present time. One mitotic figure was seen; differential counts made in several instances gave no evidence of the transformation of myelocytes into polymorphonuclear leucocytes.

Lymphocytes. These cells have a period of survival equal to that of the polymorphonuclear eosinophiles. Like them they preserve their normal character throughout their life with the exception of a rare small vacuole in the cytoplasm. In the vital preparations some show a slight increase in size and number of their normal neutral red granules. No change in size of the cell occurs. Phagocytosis is never seen. The peroxidase reaction is always negative.

Monocytes. The recognition of this cell presents the greatest difficulties after a comparatively short time of incubation. As early as forty-eight hours no typical monocytes can be found by Wright's stain. There are cells present with oval to lobulated nuclei and with cytoplasm so filled with vacuoles that its character cannot be made out. Their size is that of a monocyte. Such cells are either oxidase-negative or positive. In the vital preparations at this time the only type of cell resembling a monocyte is a cell with a round or oval nucleus and a rosette of neutral red granules. The remainder of the cytoplasm is filled with large unstained refractile granules of equal size. Phagocytosis is often present and is always at the periphery. This type of cell we have termed the "Y" cell. It can be followed for a number of days. After about a week larger forms of a similar type begin to appear and increase in number while the smaller type decreases. Whether the larger type of cell is derived from the cell supposed to be the monocyte cannot be definitely stated. A further discussion of this large type of cell is taken up below.

"X" Cells. At about the third or fourth day of incubation a type of cell appears which we have termed the "X" cell as its origin is obscure. This cell at first is about the size of a polymorphonuclear leucocyte or slightly larger. In the vital preparations it appears as a cell with one to two (sometimes four) round or oval nuclei. The cytoplasm by the neutral red method appears filled with numerous fine granules varying in color from yellow-red to brown-red; a rosette is often present, arranged around a clear centrosphere. With the Wright stain the cytoplasm varies from a gray-blue to a robin's egg blue and sometimes contains fine pinkish to purplish granules, especially in the vicinity of the nucleus. The nucleus, usually eccentric, is round with a reticulated chromatin and sometimes contains a nucleolus. Occasionally, where two nuclei are present they are connected by a delicate thread of chromatin. In both fixed and vital preparations the shape of the cell varies greatly. It may be

round, spindle-shaped, stellate or very irregular due to numerous pseudopods. Cells of this type are often phagocytic, especially the medium sized and larger forms. The phagocyted material is always situated at the periphery. By the peroxidase reaction this type of cell is strongly positive, weakly positive, or negative. Such positive granules are distributed evenly in the cell and cannot be due to phagocyted material. As the time of incubation increases the number of cells of this type becomes greater. At the same time larger forms identical in character appear. These are highly phagocytic, containing red blood corpuscles, fragments of hemoglobin and occasionally dead leucocytes. Phagocyted material, as in the smaller forms, is never placed near the nucleus but always at the periphery of the cell. This type of cell constantly lives longer than any of the other leucocytes; living cells have been found in one instance after seven weeks. Three weeks to a month is their usual time of survival. The peroxidase reaction of the larger forms is similar to that of the smaller forms — it varies from strongly positive to negative. The size of the largest cells of this type is about seven or eight times the diameter of a red blood corpuscle. The possible origin for this type of cell will be discussed below.

Large "Y" Cells. As stated under the description of the monocytes, about the seventh or eighth day there appears a large cell whose size is similar to a medium or large "X" cell. As time goes on the number and dimensions of these large cells increases, their greatest size being slightly larger than that of the largest "X" type. These cells have one to two round nuclei. In vital preparations they show a rosette of light red granules; the remainder of the cytoplasm is filled with large unstained refractile granules all of the same size. With the Wright stain the cytoplasm is filled with vacuoles. They are often highly phagocytic and, like the "X" cell, the phagocyted material is always placed at the periphery. These cells generally give a positive oxidase reaction but are occasionally negative. Their duration of life is similar to that of the "X" type and they preserve the characteristics given above throughout their survival.

Discussion

The polymorphonuclear neutrophiles and the myelocytes, as far as can be made out, had about the same period of survival and showed similar degenerative changes. That the myelocytes may have been transformed into one of the long-lived "X" or large "Y" types is possible and this would account for their apparent disappearance. The polymorphonuclear eosinophiles and the lymphocytes survived longer than the above two and neither showed any change in size or staining, except that the lymphocyte occasionally showed neutral red granules larger than normal. Neither cell was even seen to phagocyte. The two types of polymorphonuclear leucocytes and myelocytes gave a positive peroxidase reaction even when dead. The monocytes presented a difficult problem as they lost their normal characteristics so quickly. Even after forty-eight hours no typical monocytes could be found with fixed or vital preparations. In one culture in which the number of monocytes exceeded that of the myelocytes, the cells could be followed better than in the others. Here again even at forty-eight hours the only cells recognizable as monocytes were extremely vacuolated and filled with large refractile granules in the vital preparations. Such cells were generally oxidase positive. Whether this type of cell goes on to form the so-called large "Y" cell it is not possible to affirm or deny at the present time.

The "X" type of cell likewise presents a problem insoluble as yet. These cells were identical in every way with those found by Awrorow and Timofejewskij in their cultures. These authors termed them "*Ausläuferzellen*," "*Hypertrophierte Zellen*" and "*Klasmatozyten*" according to their shape and physiological activities. They believed they were all variations of the same type. They felt that the source of this type was the myeloblast of Naegeli or the lymphocyte. The "X" cell resembles no cell seen in the blood under ordinary conditions. The fact that this type appeared about the fourth day and from then on increased in number and size suggests that multiplication or transformation of other cells must have taken place. The possible origins for this cell are the myelocyte, the polymorphonuclear neutrophile or the monocyte. It cannot be an eosinophile as it is phagocytic, often peroxidase negative and never shows eosinophilic granules. Against its lymphocytic origin are its

phagocytic properties and frequently positive peroxidase reaction. In favor of the myelocyte as its progenitor are the character and arrangement of the neutral red granules in vital preparations and its occurrence in large numbers in blood rich in myelocytes. Against the myelocyte is the fact that the "X" cell is often peroxidase negative and is highly phagocytic; reversion of the myelocyte to a primitive type is a possibility. If it is derived from a polymorphonuclear neutrophile this cell must have undergone a complete transformation as regards its nucleus and cytoplasm. As many typical neutrophiles survive for a long time this explanation seems improbable. The varying peroxidase reaction, the property and method of phagocytosis and the neutral red rosette could well be accounted for by a monocytic origin. Against this, however, is the fact that monocytes even after two days incubation are filled with vacuoles. It seems hard to believe that such a cell should twenty-four to forty-eight hours later give rise to a healthy cell with a cytoplasm free from vacuoles. It is possible, of course, that a certain number of monocytes are more hardy than the others and do not undergo such vacuolization but instead differentiate into "X" cells. However, such monocytes must be extremely rare as no definite, non-vacuolated monocytes have been seen after forty-eight hours incubation. As suggested by Awrorow and Timofejewskij the myeloblast may be the parent cell, but against this is the fact that "X" cells occur in incubated normal bloods and tissues (to be reported later). A further possible source is some primitive type of cell that has the power to differentiate along different lines. Such a cell would correspond to Sabin, Doan and Cunningham's reticular cell or to Ferrata's hemohistioblast. The varying oxidase reaction, content of granules and rather primitive type of cytoplasm could be accounted for by assuming its origin from the hemohistioblast. However, hematologists are divided as to whether such cells do exist even in pathologic bloods. The reticular cell of Sabin *et al.* has not been described as occurring in the blood. Some hematologists believe that a certain percentage of the large mononuclears of the blood are endothelial in origin; such cells might be the progenitors of the "X" cells. In favor of such a view are the phagocytic powers, morphological characteristics and negative peroxidase reaction of some of the "X" cells. Opposed to such a theory is the arrangement of phagoctyed material. In an "X" cell, the pha-

gocyted material is always placed at the periphery, never next the nucleus, as described for the endothelial leucocytes (clasmatocytes) by Sabin *et al.* The positive peroxidase reaction of some of the "X" cells is also against an endothelial origin as endothelial leucocytes and the clasmatocytes of the tissues are peroxidase negative. We hope that studies now in progress will permit a more definite decision as to the source of the "X" cell.

The large "Y" cell with the neutral red rosette and numerous refractile granules may be merely a form of the "X" cell. The "Y" cell's rosette, peripheral phagocytosis, varying peroxidase reaction, size and duration of life would favor this explanation. Against the identity of the two cells is the character of the cytoplasm, the occurrence of one type alone and the time of appearance. The cytoplasm of the "Y" cell is always filled with large refractile granules. The "X" cell usually shows no such granules; if present they are smaller in size and fewer in number. In the "Y" cell the neutral red granules are light red in color, regular in size and are confined rather sharply to the rosette area. In the "X" cell the neutral red granules vary from yellow-red to brown-red in color and in size from very fine to coarse. They tend to occur throughout the cytoplasm. The finest granules are at the center of the cell and the largest at the periphery. As will be seen in the description of the lymphatic leukemia bloods either type may be present in the absence of the other. The "X" cells appear as early as the third day while the large "Y" cells are not usually seen before the seventh or eighth day. In favor of the development of the large "Y" cell from the monocyte are the similarity as regards the disposition of the neutral red, the large refractile granules in the cytoplasm, the varying oxidase reaction and the peripheral phagocytosis. The only objections to such an origin for this cell are the rapid degenerative changes and decrease in numbers of the monocyte during the early periods of incubation, while the large "Y" cell appears in increasing numbers and size from the seventh day on. The sometimes negative peroxidase reaction, the well developed phagocytic powers and the character of the cytoplasm of the large "Y" cells all argue against the myelocyte as its progenitor. The points in favor and against the derivation of the "X" cell from an endothelial cell apply also to the "Y" cell. As with the "X" cells we hope to be able to report more definite data soon.

It is impossible to say why the cells of some of the incubated bloods survived for long periods of time in the clotted blood tubes, while in others the great majority died in a comparatively short time. The number of cells alone cannot be the reason as the cells of one blood with a total leucocyte count of 17,000 lived but a short time while those of the blood with a total leucocyte count of 176,000 lived a long time. Some other factor or factors must play a part. In one experiment where the heparinized plasma was diluted several times with Locke's solution the cells survived much longer than in the undiluted plasma, suggesting that the serum in certain cases may exert an injurious influence.

It is interesting to note that sheep serum although hemolytic and agglutinative for human red blood corpuscles, apparently has very little effect on the leucocytes, since leucocytes in several instances survived for a long time in such serum.

Lymphatic Leukemia

The bloods were treated in the same way as those from the myelogenous leukemia cases.

Polymorphonuclear neutrophiles. These cells as a rule lived on an average but five days in the clotted blood tubes. In some instances all died within forty-eight hours. In the tubes where the blood was diluted the average period of survival was ten days; in one instance reaching fifteen days. The various degenerative changes observed in the myelogenous leukemic series held true here also.

Lymphocytes. In the clotted blood tubes the lymphocytes died out as rapidly and in two instances more rapidly than the polymorphonuclear neutrophiles. In the dilute tubes, however, the average time of survival was thirteen days. During this period of incubation the lymphocytes always preserved their essential characteristics, both in the fixed smears and in vital preparations. They often showed some increase in the number and size of their neutral red granules, which in arrangement frequently suggested a rosette. Such a rosette, however, could not be confused in any way with that of a monocyte owing to the shape and color of the granules. Occasionally the Wright stain showed one or two small clear vacuoles in the cytoplasm; these vacuoles were smaller and fewer in number

than those in a polymorphonuclear leucocyte or myelocyte. Some of the cells increased somewhat in size, rarely being about four times the size of a red blood corpuscle. No mitoses were seen nor were lymphocytes with more than one nucleus noted. In following the cells from day to day it was found that the percentage of small dead lymphocytes as compared to the living was always greater than that of the larger forms. The larger cells always showed a proportionately greater number of living forms, but cells of both types persisted during the period of survival.

Monocytes. In one case a few monocytes were noted in the original blood. In another a few cells, possibly monocytes, were found. After incubation, definite monocytes were present. The remainder of the cases showed no monocytes either in the original smears or after incubation. The changes shown by the monocytes during the time they could be followed were the same as in the myelogenous leukemias.

Myelocytes. In one case the original blood contained two per cent myelocytes. The myelocytes could be followed up to nine days. During this time an increasing number showed vacuolization. Their final fate is in doubt as discussed under myelogenous leukemia.

"X" Cells and Large "Y" Cells. In two cases "X" cells were found appearing in one case at the sixth day and in the other at the eighth. They were similar in appearance to those seen in the myelogenous leukemia bloods but differed in that they were apparently all oxidase-negative. In one case several of the cells showed foci of peroxidase positive granules but from their distribution and arrangement it was obvious that these represented phagocyted polymorphonuclear leucocytes. Large "Y" cells were found also in two cases, one of these being that in which the "X" cells were found. Their characteristics given above under myelogenous leukemia apply here also. Their peroxidase reaction varied from positive to negative. Their periods of survival were seventeen days to thirty-four days. In no case did the number of the "X" cells or the large "Y" cells approach that found in the myelogenous leukemia bloods.

Discussion

The most striking fact in regard to these bloods was the failure of the cells to survive in the clotted blood tubes. All types of cells apparently died in a comparatively short period of time. The average period of survival was five days although it was at times as short as forty-eight hours. The contrast of the period of survival of the lymphocytes in the myelogenous series to that in this series is striking, the average period in the former being twenty days or four times as long. The polymorphonuclear neutrophiles in the myelogenous bloods survived in clotted blood usually about two weeks or three times as long as in this series. In the diluted bloods the lymphocytes survived on an average nineteen days and the polymorphonuclear neutrophiles eleven days, a marked contrast to the results in the clotted blood. At first it was thought that the number of cells in the clotted bloods was the reason but apparently this could not be the sole explanation as in one case the total count was 2,500 and the cells died in forty-eight hours. Also, in a myelogenous leukemia case where the total count was 196,000 the lymphocytes survived over two weeks in clotted blood. Some other factor must be involved.

It is interesting to note that whereas the "X" cells in the myelogenous series varied from strongly peroxidase-positive to negative, the "X" cells seen in this series were oxidase-negative. The cells from both series were morphologically identical. It seems improbable that the lymphocyte is the parent cell of the "X" cell in these bloods for throughout the period of incubation the lymphocytes always preserved their normal appearance and within limits their size; they showed no transitions to the other type. The large "Y" cells appeared in the two cases in which monocytes were present; this occurrence may be of some significance. In one of these cases myelocytes were present also. The period of survival of both the "X" cells and large "Y" cells was about the same as in the myelogenous series.

Summary and Conclusions

Blood from eight cases of myelogenous leukemia and seven cases of lymphatic leukemia was incubated at 37.5° C. Such bloods were incubated as clotted blood and in addition were diluted in different ways. Specimens were removed at varying periods and were studied

supravitally and in fixed smears. In five of the cases of myelogenous leukemia the cells in the clotted blood tubes survived longer than in the diluted blood tubes but in the lymphatic leukemia cases in no instance did the cells survive as long in the clotted blood as in the dilute tubes.

In the myelogenous series the myelocytes and polymorphonuclear leucocytes lived, on an average, two weeks. The eosinophiles and lymphocytes survived about three weeks. The monocytes showed early vacuolization and were followed with difficulty after the fourth day. They may have become the large "Y" cells.

In the lymphatic leukemia series the lymphocytes and polymorphonuclear leucocytes lived, on an average, five days in clotted blood. In diluted blood the lymphocytes survived nineteen days and the neutrophiles eleven days. Monocytes were found in but two cases and showed the same degenerative changes as in the myelogenous series. In one case myelocytes were found and could be followed for nine days.

In all the myelogenous leukemia cases and in two of the lymphatic series two types of cells appeared that are not seen in blood under ordinary conditions. The first, called by us the "X" cell, appeared usually about the fourth day and increased in size and number for several days. The longest time of survival was seven weeks. These cells have round nuclei and basophilic cytoplasm, often with pink or purplish granules; their shape is irregular; they vary from strongly oxidase-positive to negative and they are phagocytic. In the supravital preparation they show numerous fine neutral red granules throughout the cytoplasm but also often in addition in a rosette form, frequently with a clear centrosphere; phagocytosis is always at the periphery of the cell.

The other type of cell, the large "Y" cell, has one to two round to oval nuclei and bluish cytoplasm with numerous vacuoles; is usually oxidase-positive, sometimes negative; in the vital preparations it has a large rosette of neutral red and the surrounding cytoplasm is filled with large refractile granules; phagocytosis is always at the periphery; the shape of this cell is usually round. The origin of both these types of cells is at present undetermined.

The methods employed would seem to be suitable for the study of the effect of different substances or conditions on the normal and tumor cells of the blood.

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